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<p>(71) Applicants: <b>INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM) [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cédex 13 (FR). CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS) [FR/FR]; 3, rue Michel-Ange, F-75794 Paris Cédex 16 (FR). UNIVERSITE LOUIS PASTEUR, STRASBOURG 1 [FR/FR]; 4, rue Blaise Pascal, Boîte postale 1032/F, F-67070 Strasbourg Cédex (FR). E.R. SQUIBB &amp; SONS, INC. [US/US]; Lawrenceville-Princeton Road, Princeton, NJ 08543-4000 (US).</b></p> <p>(72) Inventors: <b>BENOIST, Christophe, O.; 2, rue des Hallebardes, F-67000 Strasbourg (FR). MATHIS, Diane, J.; 2, rue des Hallebardes, F-67000 Strasbourg (FR). KOUSKOFF, Valerie; National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206 (US).</b></p>		<p><b>Published</b>  <i>Without international search report and to be republished upon receipt of that report.</i></p>

Figure 1: Schematic diagram of the pGEM-10.00 plasmid and its DNA sequence. The plasmid is circular, 10.00 kb, with a multiple cloning site (MCS) containing 11 restriction sites. The MCS is flanked by a 'PORTABLE REGION' and a 'CONSTANT REGION'. The sequence is shown as a linear map with restriction sites labeled: SalI 19.00, ClaI 19.00, SalI 20.00, BamHI 0.00, HindIII 2.18, EcoRV 2.34, PstI 2.63, HindIII 2.90, EcoRI 3.50, BamHI 5.00, EcoRI 6.22, EcoRI 12.30, BamHI 12.50, EcoRI 12.50, BamHI 14.50, SalI 15.20, EcoRI 15.00, ClaI 19.00, SalI 20.00.

A transgenic animal model for arthritis is disclosed. The arthritic condition results from genetic (or immunologic) manipulations that result in the T cell receptor (TCR) repertoire of the animal being substantially limited relative to the TCR repertoire of a wildtype control animal. The TCR repertoire of the arthritic animal, albeit limited, is functionally viable. In a preferred embodiment, the invention relates to transgenic arthritic mice wherein arthritis results from (1) a transgenic allele which encodes and expresses TCR  $\alpha$  and  $\beta$  subunits that combine in T lymphocytes of the transgenic animal to form a TCR that recognizes an antigen comprising one or more epitopes of an oligopeptide derived from amino acids 41-61 of bovine pancreatic ribonuclease and/or (2) polypeptide arthritogenic self antigens derived from endogenous proteins. The transgenic arthritic mice of the invention provides an animal model which faithfully mimics rheumatoid arthritis and by which human arthritogenic and therapeutic anti-arthritic compositions are evaluated. Also provided herein are therapeutic oligopeptides derived from the variable regions of the TCRs of the transgenes of the invention and/or from the amino acid sequence of proteins comprising endogenous polypeptide arthritogenic antigens.

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**Transgenic Arthritic M*****Field of the Invention***

The present invention relates to transgenic non-human animals that have been genetically engineered to develop arthritis and thus provides animal models for human arthritic diseases such as Rheumatoid Arthritis. More particularly, the invention relates to transgenic animals wherein the T cell receptor (TCR) populations of the transgenic animals consist substantially of copies of a TCR that (a) comprises transgene encoded TCR $\alpha$  and TCR $\beta$  subunits and (b) directs T cells to react with self antigens in the transgenic animals. Most particularly, the invention relates to transgenic animals wherein a limited TCR repertoire, consisting substantially of transgene encoded TCRs, acts during development of the transgenic animal to initiate a cascade of events that culminates in the transgenic animal developing severe arthritic symptoms in a reproducible and thus predictable manner.

***Background of the Invention***

Animals have a complex array of molecular and cellular defenses, collectively referred to as the immune system, that recognize and attack potentially harmful foreign or endogenous but abnormal cells (respectively represented by, e.g., pathogens such as bacteria or viruses, and cancerous or pathogen-infected cells), but that do not attack but rather tolerate endogenous normal cells. When stimulated by foreign or abnormal biomolecules, the immune system undergoes a series of activities designed to neutralize and destroy the pathogens, or cancerous or pathogen-infected cells, with which the foreign or abnormal biomolecules are associated. These activities, collectively known as an immune response, may consist of a cell-mediated immune response, a humoral (antibody-mediated) immune response, or an immune response that includes elements of cell-mediated and humoral responses.

Humoral immune responses are mediated by antibodies, glycoproteins that bind specific foreign or abnormal biomolecules and attract other

components of the immune system thereto. Antibodies are immunoglobulin (Ig) molecules produced by B cells, lymphocytes which originate in avian bursa or in mammalian bone marrow but migrate to and mature in other organs, particularly the spleen. Robertson, M., *Nature* 301:114 (1983). Cell-mediated immune responses are the result of activities of T cells, lymphocytes that undergo maturation within the thymus of an animal. Tizard, p. 163.

T cell activities vary considerably among different subpopulations of T cells within an animal. Cytotoxic T cells recognize and destroy foreign cells (graft rejection) or endogenous but abnormal cells (e.g., cancerous cells or cells infected with intracellular parasites such as viruses and bacteria). Helper T cells interact with, and produce biomolecules that influence the behavior of, both B cells and cytotoxic T cells, in order to promote and direct antibody production and cytotoxic activities, respectively. Mosier, D.E., *Science* 158:1573-1575 (1967). Other classes of T cells, including suppressor T cells and memory T cells, also exist. Miedema, F., and Melief, C.J.M., *Immunol. Today* 6:258-259 (1983); Tizard, I.R., *Immunology: An Introduction*, Saunders, Philadelphia (1988), pp. 225-228. Classes of T cells are to some extent distinguished on the basis that different T cells display different CD proteins on their surfaces. Immature T cells display both CD4 and CD8 proteins (i.e., immature T cells are CD4<sup>+</sup>8<sup>+</sup>), mature helper T cells are CD4<sup>+</sup>8<sup>-</sup> (i.e., display CD4 protein but not CD8 protein) and mature cytotoxic T cells are CD4<sup>-</sup>8<sup>+</sup> (i.e., display CD8 protein but not CD4 protein). Smith, L., *Nature* 326:798-800 (1987); Weissman, I.L., and Cooper, M.D., *Sci. American* 269:65-71 (1993).

In order to function properly, the T and B cells of an animal's immune system must accurately and reliably identify an enormous number of molecular "non-self" compositions, i.e., compositions that are either foreign or endogenous but abnormally expressed. Recognition and identification by the immune system occurs at the molecular level. An antigen, a molecular composition having the potential to generate an immune response, is composed of one or more molecular-sized identifying features known as epitopes. A



polypeptide antigen which has an amino acid sequence which comprises, e.g., a hundred amino acids might comprise dozens of epitopes, wherein each epitope is defined by a portion of the polypeptide comprising from about 3 to about 15 amino acids. The number of epitopes derivable from polypeptides alone is estimated to be about ten million. Tizard, p. 25.

An antigen encountered by a T or B cell of an animal must be identified as either being associated with normal endogenous (i.e., self) antigens, an immune response to which would be injurious to the animal, or with foreign or abnormal (i.e., non-self) antigens, to which an immune response should be mounted. The process can be analogized to "friend or foe" identification in human combat. If the immune system fails to identify antigens associated with invading pathogens or tumor cells as non-self, then these "enemies" can slip through the system's defenses. If the immune system mistakenly identifies an animal's endogenous antigens as non-self, then the parts of the animal's body that comprise the endogenous antigens will face "friendly fire" from the immune system. The latter situation, in which an animal's immune system mistakenly wages cellular and molecular "war" against another, normal part of an animal's body, is known generally as "autoimmune disease."

As part of the immune system's means of identifying antigens, individual T and B cells produce antigen receptors which are displayed on the T or B cell's surface and which bind specific antigens. The T cell receptors (TCRs) produced by and displayed on an individual T cell comprise heavy (TCR $\beta$ ) and light (TCR $\alpha$ ) polypeptide subunits. Each TCR  $\alpha$  and  $\beta$  subunit has a carboxy-terminal constant region, the amino acid sequence of which does not vary from T cell to T cell, and an amino-terminal variable region, the amino acid sequence of which does vary from T cell to T cell. When TCR $\alpha$  and TCR $\beta$  subunits associate with each other, the variable regions of the TCR $\alpha$  and TCR $\beta$  polypeptide subunits combine to form the unique antigen-binding portion of the TCR. Davis, M.M., and Bjorkman, P.J., *Nature* 334:395-404 (1988). Similarly, individual B cells produce and display

antigen receptors that comprise Ig molecules which have unique antigen-binding portions due to unique amino acid sequences in the variable regions of each of the two antibody subunits, known as the Ig heavy and Ig light chains. Each B cell membrane comprises from 20,000 to 200,000 identical Ig molecules. Tizard, pp. 78-80 and 202.

Although each individual T or B cell displays identical antigen receptors, an animal's collection of different antigen receptors is quite diverse. The variable region of an Ig heavy chain, or that of a TCR $\beta$  chain, is encoded by three gene segments, the variable (V), diversity (D) and joining (J) segments. The variable region of an Ig light chain, or that of a TCR $\alpha$  chain, is encoded by V and J gene segments. Multiple DNA sequences encoding many different V, D and J gene segments are present as unexpressed copies in germline DNA; an analogous but different collection of variable gene segments for TCR subunits is also present. During development of an animal, genes encoding diverse variable regions are generated in individual cells of the immune system by the random joining of V, D and J, or V and J, gene segments. The process of DNA rearrangements that generates a randomly assembled variable region of an Ig heavy or TCR $\beta$  subunit is called V-D-J joining; the analogous process that generates a rearranged variable region of an Ig light or TCR $\alpha$  subunit is called V-J joining. Sakano, H., *et al.*, *Nature* 280:288-294 (1979); Early, P., *et al.*, *Cell* 19:981-992 (1980); Alt, F.W., *et al.*, *Science* 238:1079-1087 (1987); Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988), pages 10-18; Davis, M.M., and Bjorkman, P.J., *Nature* 334:395-404 (1988). Additionally, point mutations are introduced throughout the variable region by a process called somatic mutation. Bernard, O., *et al.*, *Cell* 15:1133-1144 (1978).

A functionally rearranged Ig or TCR subunit gene is one in which the DNA rearrangements of V-D-J or V-J joining and/or somatic mutation have not resulted in a reading frame that is prematurely terminated during biosynthesis because of the introduction of stop codons or frameshifting mutations. Because each T or B cell of the immune system expresses genes

encoding their respective antigen receptors in which a unique functionally rearranged variable region is present, many different T or B cells, each producing a receptor that has a unique antigen-recognizing region, are generated. The total catalog of different antigen receptors displayed on the T cells of an animal is referred to as the animal's TCR repertoire. Bevan, M.J., *Science* 264:796-797 (1994).

For mature T or B cells, binding of antigen to a cell's antigen receptor activates the cell, i.e., stimulates the cell to undertake activities related to generating a cell-mediated or humoral immune response. In contrast, for immature T or B cells, binding of antigen to a displayed TCR or B cell antigen receptor, respectively, results in elimination of the cell by a process called negative selection or clonal deletion. Clonal deletion occurs during normal development of a healthy wildtype animal, and is a mechanism by which the immune system learns to tolerate the animal's normal endogenous (self) antigens, i.e., to treat the animal's self antigens as non-immunogenic antigens. Failure of the immune system to achieve or maintain tolerance of self antigens may result in autoimmune responses (i.e., autoimmune response to self antigens) that can culminate in autoimmune disease in animals including humans. Autoimmune disease can occur when an appropriate immune response to a non-self antigen results in the production of immune effector biomolecules (e.g., autoantibodies) or cells that cross-react with self antigens. Human autoimmune diseases include such crippling conditions as Multiple Sclerosis (MS) and Systemic Lupus Erythematosus (SLE). For a review, see Steinman, L., *Sci. American* 269:107-114 (1993).

Although B cells can directly bind soluble antigen, T cells respond to antigen only when it is displayed on specific classes of other cells known generically as antigen-presenting cells (APCs). APCs, e.g., macrophages and dendritic cells, present antigens derived from polypeptides via glycoproteins, known as MHC (major histocompatibility complex) proteins, which are displayed on the surface of APCs. Bevan, M.J., *et al.*, *Science* 264:796-797 (1994). MHC proteins bind antigens generically; the specificity

determinants in a TCR:Ag:MHC complex are (1) the unique polypeptide sequences of the variable portion of the TCR and (2) the unique polypeptide sequences of antigen. However, to some degree, MHC-presented oligopeptide antigens are embedded within an MHC molecule and TCR recognition of antigen only occurs within the context of an appropriate class of MHC molecule. Janeway, C.A., *Sci. American* 269:73-79 (1993). This phenomenon, called MHC restriction, is of fundamental importance to T cell antigen recognition and physiology. Zinkernagel, R.M., and Doherty, P.C., *Nature* 248:701-702 (1974).

The genes encoding MHC proteins are diverse; however, unlike Ig and TCR molecules, which vary from cell to cell in an individual animal, MHC antigens vary from individual animal to individual animal or from one group of related individual animals to another group. Members of familial groups, represented in the mouse by inbred strains of mice, share similar MHC antigens with each other, but not with individuals from other strains of mice. Snell, G.D., *Science* 213:172-178 (1981). Because variant MHC molecules will be capable of binding different antigens, the antigens that T cells will be able to recognize (i.e., bind in the MHC context) and respond to varies among different strains of mice. In humans, particular MHC variant molecules are more highly associated with autoimmune diseases, presumably because these MHC variant molecules are more competent at binding (and thus presenting to T cells) self antigens. Vaughan, in *Immunological Diseases*, 3rd Ed., Vol. II, Samter, M., ed., pp. 1029-1037 (1978); Steinman, L., *Sci. American* 269:107-114 (1993).

Arthritis is a painful manifestation of many human autoimmune diseases. The etiology of arthritis is, in most cases, unknown. Inflammatory lesions of synovial joints occur in general autoimmune diseases such as SLE, but are more frequently present in specific autoimmune diseases such as Rheumatoid Arthritis (RA) or Reactive Arthritis (ReA). RA, the prototypic arthritic disease, occurs in an otherwise globally normal immunological context. RA is a chronic inflammatory disease that is characterized by the

development of one or more synovial membranes into a highly vascularized tissue known as the pannus. The pannus consists of several distinct types of cells, including resident synovial fibroblasts as well as infiltrating mononuclear cells that can produce inflammatory effector and mediator biomolecules. The pannus exhibits invasive growth into surrounding tissues with a corresponding progressive destruction of articular cartilage and bone. Harris, E.D., Jr., in *Textbook of Rheumatology*, Kelly, W., et al., Eds., W.B. Saunders Co., Philadelphia, pages 905-942 (1989).

Symptoms of rheumatoid arthritis range from swelling of one or more joints and associated pain to comparatively mild morning stiffness affecting one or more joints of the limbs. In severe cases, chronic inflammation results in destruction and deformity of the cartilage and bone in the joints, accompanied by chronic pain and physical deformation of the affected limbs. Chronic pain and deformation have obviously serious consequences in regard to the loss of joint mobility and quality of life of affected individuals. Generally, 50% of patients with RA can lead fairly normal lives; about 25% are partially incapacitated, with gradually progressing disability; and about 25% lead greatly restricted lives. A small proportion (less than 10%) of RA patients are totally disabled. Christian, C.L., and Paget, S.A., "Rheumatoid Arthritis" in *Immunological Diseases*, Vol. II, 3rd Ed., Samter, M., Ed., Little, Brown and Co., pages 1061-1076 (1978).

RA is a frequent human disease, affecting close to 1% of the human populations of developed countries, which has a complex component of genetic predisposition. Wordsworth, P., *Current Opinion in Immunology* 4:766-769 (1992). RA is also observed in domestic animals, especially dogs. Tizard, pp. 527-528. Some studies indicate that, in humans, RA is two to three times more common in females than in males. Christian, C.L., and Paget, S.A., "Rheumatoid Arthritis" in *Immunological Diseases*, Vol. II, 3rd Ed., Samter, M., Ed., Little, Brown and Co., pages 1061-1076 (1978). It has been suggested that the female hormone estrogen stimulates DNA sequences that indirectly induce the production of  $\gamma$ -interferon, which stimulates the

appearance of human MHC molecules in synovial joint linings, where they are not normally found. Fox, H.S., *et al.*, *J. Immunol.* 146:4362-4367 (1991).

The cause of rheumatoid arthritis is currently unknown and, in the absence of clear evidence, the subject of debate. The disease develops as follows. Histologically, the affected joints are infiltrated by cells of the hemopoietic lineage (lymphocytes, macrophages, and/or neutrophils) which produce inflammatory cytokines. The synovial lining itself is hyperplasmic and forms, together with inflammatory cells, a granuloma-like "pannus" which erodes and invades the cartilage and bone. Enzymes derived from the exudative and proliferative processes of inflammation hydrolyze various substrates in cartilage, bone, ligaments and tendons, resulting in structural deformation of synovial joints. As RA progresses, the infiltrating neutrophils may be supplemented or partially replaced by CD4<sup>+</sup> lymphocytes, which can form lymphoid nodules and germinal centers. Rheumatoid factors, autoantibodies of the IgM class directed to antibodies of the IgG class, are present in the sera of some but not all patients. Christian, C.L., and Paget, S.A., "Rheumatoid Arthritis" in *Immunological Diseases*, Vol. II, 3rd Ed., Samter, M., Ed., Little, Brown and Co., pages 1061-1076 (1978).

Although the terminal events of the pathogenesis of RA are relatively well understood or at least described, the cellular and molecular causes of initiating and early events of RA have not been described in detail. For example, both cytotoxic and humoral immune responses have been described in previously known RA models. Chiochia, G., *et al.*, *J. Immunol.* 145:519-525 (1990). Indeed, whether RA represents an immune response to self antigens with secondary inflammatory consequences, or a primary dysregulation of macrophage function, has been a matter of debate. Firestein, G.S., and Zwaifler, N.J., *Arthritis and Rheumatism* 33:768-773 (1990); Lanchbury, J.S., and Pitzalis, C., *Current Opinion in Immunology* 5:918-924 (1993). Although some candidate self antigens have been proposed for RA, none has been conclusively identified. Sano, H., *et al.*, *J. Cell Biol.* 110:1417-1426 (1990).

Current animal models of RA include the following immunologic models. For a review, see Schwartz, R.S., in *Fundamental Immunology*, 3rd Ed., Paul, W.E., ed., Raven Press, N.Y., pages 1064-1067 (1993).

5           1.       Collagen induced arthritis (CIA) seems mainly dependent on autoantibodies. It is induced by immunization of rats or mice with type II collagen (the type that is prevalent in joint cartilage), or by administration of antibodies to type II collagen. Collagen-specific T helper cells can also transfer the disease. IgG antibodies are concentrated in the synovial fluids and form deposits in the joint, presumably leading to inflammation. A similar  
10       rabbit model exists but is less well-characterized. Jasin, H.E., *Fed. Proc. (USA)* 32:147-152 (1973).

          2.       Adjuvant arthritis is induced in susceptible strains of rats by injection of *Mycobacterium tuberculosis* in oil. Joint inflammation and swelling of the joints ensues, predominantly in the distal parts of the limbs,  
15       which is transitory and disappears after 21 days, leaving sequellar destruction. Pearson, C.M., *J. Chronic Dis.* 16:863-874 (1963). In this case, the disease is transferable from rats with disease to naive animals with T lymphocyte populations or lines. It appears that the T cells and/or antibodies elicited by immunization with *M. tuberculosis* may be cross-reactive between  
20       proteoglycan structures in the bacteria and cartilage. Pristane-induced arthritis (PIA) is similarly induced by injection of the drug pristane in mineral oil. Potter, M., and Wax, J.S., *J. Immunol.* 127:1591-1595 (1981).

          3.       *Lpr/fas* arthritis occurs in mice of the MRL/*lpr* strain which carry a mutation in the *fas* gene, involved in the induction of cell death  
25       through apoptosis. As a result of this deficiency, a massive lymphoproliferation occurs in these mice, primarily composed of an unusual population of T lymphocytes. Several autoimmune manifestations result, including progressive inflammation of joints. Cheng, J., *et al.*, *Science* 263:1759-1762 (1994).

30       None of these models is quite satisfying. In CIA, adjuvant arthritis and PIA, the animal is artificially immunized with antigens that result in an

immune response that cross-reacts with cartilage-borne biomolecules; for example, all three form of arthritis can be treated by teaching the immune system tolerance for type II collagen. Thompson, H.S.G., and Staines, N.A., *Clin. Exp. Immunol.* 64:581-586 (1985); Zhang, Z.J., *et al.*, *J. Immunol.* 145:2489-2493 (1990); Thompson, S.J., *et al.*, *Immunol.* 79:152-157 (1993). However, destruction of cartilage-associated structures occurs late in RA, possibly as a secondary effect, so these models likely do not reflect arthritis in which primary or early events in the immune system result in a cascade of autoimmunological events that culminates in arthritis. *Lpr/fas* arthritis is a lymphoproliferative disease with only secondary arthritic consequences, a setting that is clearly different from the globally normal immunological context of RA. An animal model that incorporates the method of CIA into an autoimmune disease prone background results in more severe arthritic symptoms but suffers from both of the above drawbacks. Watson, W.C., *et al.*, *J. Exp. Med.* 172:1331-1339 (1990).

Moreover, none of the above immunologic methods provides an animal model in which the animal develops severe arthritic symptoms in a reproducible and thus predictable manner, thus allowing the characterization and manipulation of early immunologic events in RA. Recently, the ability to produce transgenic animals has provided an opportunity to develop animal models that more faithfully reflect human diseases, including autoimmune diseases. Watson, J.D., *et al.*, "The Introduction of Foreign Genes Into Mice," in *Recombinant DNA*, 2d Ed., W.H. Freeman & Co., New York (1992), pp. 255-272; Lee, M.-S., and Sarvetnick, N., *Curr. Op. Immunol.* 4:723-727 (1992). A transgenic animal is an animal into which has been introduced by unnatural means (i.e., by human manipulation), one or more genes that do not occur naturally in the animal, e.g., foreign genes, genetically engineered endogenous genes, etc. The unnaturally introduced genes, known as transgenes, may comprise foreign DNA sequences, i.e., sequences not normally found in the genome of the host animal. Alternatively or additionally, transgenes may comprise endogenous DNA sequences that are



abnormal in that they have been rearranged or mutated *in vitro* in order to alter the normal *in vivo* pattern of expression of the gene, or to alter or eliminate the biological activity of an endogenous gene product encoded by the gene. Watson, J.D., *et al.*, "The Introduction of Foreign Genes Into Mice," in *Recombinant DNA*, 2d Ed., W.H. Freeman & Co., New York (1992), pp. 255-272; Gordon, J.W., *Intl. Rev. Cytol.* 115:171-229 (1989); Jaenisch, R., *Science* 240:1468-1474 (1989); Rossant, J., *Neuron* 2:323-334 (1990).

In the course of investigating the immune system, scientists have generated several transgenic TCR animals, i.e., animals which comprise a transgene that directs the expression of a particular TCR or TCR subunit. Krimpenfort, P., *et al.*, *EMBO Journal* 7:45-50 (1988); Teh, H.S., *et al.*, *Nature* 335:229-233 (1988); Bluthmann, H., *et al.*, *Nature* 334:156-159 (1988); Uematsu, Y., *et al.*, *Cell* 52:831-841 (1988); Steinmetz, M., *et al.*, *Genome* 31:652-655 (1989); Berg, L.J., *et al.*, *Cell* 58:1035-1046 (1989); Uematsu, Y., *Eur. J. Immunol.* 22:603-606 (1992); Borgulya, P., *et al.*, *Cell* 69:529-537 (1992); Katz, J.D., *et al.*, *Cell* 74:1089-1100 (1993). Introduction of a transgenic TCR "knockout" (loss of function) allele into mice results in animals which are substantially depleted for T cells. Krimpenfort, P.J.A., and Berns, A.J.M., U.S. Patent 5,175,384 (Dec. 29, 1992). More subtle biological effects result from the introduction of transgenic TCR alleles which confer altered specificities to an animal's T cells. However, the introduction of a transgenic TCR allele derived from a T cell clone capable of transferring collagen-induced arthritis into mice does not result in arthritis in the resultant transgenic animals. Mori, L., *et al.*, *J. Exp. Med.* 176:381-388 (1992).

The references discussed above and elsewhere herein are provided solely for the disclosure prior to the filing date of the present application and nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosures by virtue of prior invention.

Given the state of the art, a need exists for animal models in which the animal develops severe arthritic symptoms in a reproducible and thus

predictable manner, in order to characterize and control early immunologic events in autoimmune arthritis, particularly rheumatoid arthritis. Accordingly, it is an object herein to provide transgenic non-human animals that have been genetically engineered to develop autoimmunological arthritis by virtue of the fact that the T cell receptor (TCR) populations of the transgenic animals consist substantially of copies of a TCR that (a) comprises transgene encoded TCR $\alpha$  and TCR $\beta$  subunits and (b) directs T cells to react with self antigens during development of the transgenic animals to initiate a cascade of autoimmunological events that culminates in the transgenic animals developing severe arthritic symptoms in a reproducible and thus predictable manner.

It is also an object herein to provide materials and methods for making transgenic non-human arthritic animals, some of which can be used to generate therapeutic anti-arthritic compositions.

It is a further object herein to provide methods of using the transgenic non-human arthritic animals to evaluate the anti-arthritic or arthritogenic potential of various compositions of matter, including animal and human genes and the gene products encoded thereby.

Further, it is an object herein to provide hybridomas derived from B or T cells of the transgenic non-human arthritic animals, and specific biomolecules produced in association with transgene associated arthritis by the hybridomas and the B and T cells from which they are derived.

Still further, it is an object herein to provide an isolated protein endogenous to animals including humans which comprises polypeptide arthritogenic self antigens, methods of purifying or producing the isolated protein, and methods of using the isolated protein or compositions derived therefrom to treat human and animal arthritic diseases.

### *Summary of the Invention*

The invention is based on the unexpected discovery that transgenic non-human animals having a limited TCR repertoire that consists substantially of transgene encoded TCRs have a phenotype that selectively comprises severe classical symptoms of rheumatoid arthritis. The TCR repertoire of the  
5 arthritic animal, albeit limited, is functionally viable. The limited TCR repertoire of the arthritic animal consists in substantial part of a class of TCRs that recognize an epitope of an endogenous polypeptidic arthritogenic antigen. In a preferred embodiment, the invention relates to transgenic arthritic mice  
10 wherein an arthritic phenotype is present due to the insertion into the genomes of the mice of transgenes encoding TCR  $\alpha$  and  $\beta$  subunits that combine in T cells of a transgenic animal to form a TCR that recognizes an antigen comprising one or more epitopes of an oligopeptide corresponding to amino acids 41-61 of bovine pancreatic ribonuclease (BPR) and having the amino  
15 acid sequence

Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-

Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1],

and/or one or more epitopes of an endogenous polypeptide arthritogenic self antigen that are substantially cross-reactive with those of the BPR-derived  
20 oligopeptide.

In one aspect, the invention provides an animal model for arthritis. Because of the crippling and painful nature of arthritis in affected individuals, as well as its economic consequences to society, it is desired to obtain the benefit of a reliable and reproducible animal model of arthritis. The  
25 transgenic animal model of the invention provides a model in which the animals develop severe arthritic symptoms in a reliable and reproducible manner. The reliability and reproducibility of the transgenic animal model of the invention are not found in previously known animal models of arthritis.

In a related aspect, the invention provides (1) methods for producing a transgenic non-human animal which has a limited TCR repertoire due to the expression therein of transgenes encoding TCR  $\alpha$  and  $\beta$  subunits and (2) means for generating the transgenic animals of the invention, including isolated DNA molecules capable of expressing DNA sequences encoding the variable and constant regions of the  $\alpha$  and  $\beta$  subunits in T cells of a transgenic animal in order to direct the formation of a TCR that recognizes an antigen comprising one or more epitopes of an oligopeptide corresponding to amino acids 41-61 of bovine pancreatic ribonuclease and having the amino acid sequence

Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-

Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1],

and/or one or more epitopes of an endogenous polypeptide arthritogenic self antigen. Oligopeptides derived from the amino acid sequence of the TCR variable regions of the invention are used to design therapeutic compositions.

In another aspect, the invention provides methods of using the animal model of the invention to (1) test compositions for their anti-arthritic (therapeutic) potential, and (2) evaluate compositions for their arthritogenic (hazardous) potential.

The transgenic animal of the present invention is also useful as a source of immune system cells and biomolecules that recognize endogenous polypeptide arthritogenic self antigens (PASAs). Hybridomas derived from lymphocytes from transgenic arthritic mice of the invention produce immunologic effector biomolecules that specifically recognize endogenous proteins comprising PASAs. These immune system cells and biomolecules are used to assay the concentration and distribution of PASA-comprising proteins in animals including humans.

Utilizing the one or more of the mice or cell lines of the present invention, or immunologic effector biomolecules produced thereby, the present

invention further provides methods of identifying and purifying endogenous proteins comprising PASAs from animals including humans. Thus, in another aspect, the invention provides isolated endogenous proteins comprising PASAs, and, in a related aspect, the invention provides for oligopeptides derived from the isolated endogenous proteins comprising PASAs. Means and methods of producing the isolated endogenous proteins comprising PASAs from biological fluids from animals including humans, as well as by expression through recombinant DNA technology, are also provided.

In yet another aspect, the invention provides therapeutic compositions for rheumatoid arthritis comprising isolated endogenous proteins comprising PASAs, one or more isolated oligopeptides comprising amino acid sequences derived from the polypeptide sequence of the endogenous proteins comprising PASAs, or one or more synthetic polypeptides derived from the amino acid sequences of the variable regions of the TCR subunits expressed by hybridoma R28.

### *Brief Description of the Drawings*

Figure 1 depicts the plasmids used to construct KRN transgenic mice. Panel A of Figure 1 depicts paKRN, which is composed of DNA sequences from a prokaryotic vector, pEMBL18 (thick line) and approximately 16 kb of DNA sequences from the functionally rearranged TCR $\alpha$  allele from hybridoma R28. The exons (coding sequences) of the TCR $\alpha$  subunit are stippled, and the direction of their transcription is indicated by arrows; non-coding sequences (introns and sequences flanking the exons) are shown by thin lines. The DNA sequence of the exon encoding the variable region of the TCR $\alpha$  subunit is given in the inset; note that a short intron which interrupts the coding sequence of the variable region but which is excised in mature mRNA is not shown on the plasmid diagram for the sake of clarity. Panel B of Figure 1 depicts pbKRN, which is composed of DNA sequences from a prokaryotic vector, pTZ18 (thick line) and approximately 18 kb of DNA

sequences from the functionally rearranged TCR $\beta$  allele from hybridoma R28. Other symbols are as in Panel A of Figure 1, except that the exons encoding the TCR $\beta$  subunit are hatched.

5 Figure 2 depicts a nucleic acid hybridization ("Southern") assay for presence of DNA sequences of the KRN transgenic allele among offspring produced from a cross of KRN transgenic animal with a non-transgenic animal. A KRN transgenic female (filled circle) was crossed with a non-transgenic male (empty square), and DNA samples isolated from the tails of resulting progeny were probed with detectably-labelled transgene-specific  
10 DNA sequences.

Figure 3 compares the results of cytofluorimetric analyses of the expression of T cell receptors in a wildtype mouse (left) with results obtained from examination of a KRN transgenic mouse (right). In Panel A of Figure 3, cells from the lymph node of the mice were stained with polyclonal  
15 antibodies directed to an epitope composed of aligned portions of the TCR $\alpha$  and TCR $\beta$  subunits of hybridoma R28. In Panel B of Figure 3, cells were stained with a monoclonal antibody specific for the V $\beta$ 6 portion of the variable region used in the gene for the TCR $\beta$  subunit of hybridoma R28 and other functionally rearranged TCR $\beta$  genes.

20 Figure 4 shows the clinical manifestations of arthritis in transgenic arthritic mice and the course of development of arthritis therein. In Panel A of Figure 4, the hind limb of a transgenic KRN x NOD arthritic mouse is shown adjacent to the hind limb of a non-transgenic littermate. Panel B of Figure 4 is a chart displaying the results of chronological measurements of  
25 ankle thickness of transgenic KRN x NOD arthritic mice and their non-transgenic littermates.

Figure 5 demonstrates the arthritic phenotype induced by the KRN transgenic allele in different genetic backgrounds. Three crosses are shown; in each case a male KRN transgenic animal (filled squares) derived from the  
30 B6 strain of mice was crossed with a non-transgenic female animal (open circles) of various strain backgrounds. In the cross diagrammed in the Top

Panel of Figure 5, the non-transgenic female animal is from the B6 strain of mice, and no arthritis is observed in the offspring. In the cross shown in the Middle Panel of Figure 5, the non-transgenic female animal is from the NOD strain of mice, and complete penetrance of the arthritic phenotype occurs, evidenced by the fact that all transgenic offspring develop arthritis. In the cross shown in the Bottom Panel of Figure 5, complete penetrance of the arthritic phenotype is also seen when the non-transgenic female animal has a cogenic genome which includes the MHC loci of the NOD strain (B6.H2<sup>nod</sup>).

Figure 6 contrasts a radiograph (X-ray image) of the hind limb of a transgenic KRN x NOD arthritic mouse (Right Panel) with a radiograph of the hind limb of a non-transgenic, non-arthritic littermate (Left Panel).

Figure 7 shows the effect of anti-CD4 monoclonal antibody in preventing the occurrence of arthritis in transgenic arthritic mice, compared to the occurrence of arthritis in untreated control transgenic arthritic mice. The progress of arthritis was determined by clinical examination of the animals every third day.

### *Detailed Description of the Disclosure*

#### *Terms and Symbols*

For purposes of this disclosure, the following abbreviations, definitions, genetic designations and restriction enzyme recognition sequences are used herein unless otherwise indicated.

**TABLE 1. ABBREVIATIONS**

Ag	=	antigen
APC	=	antigen presenting cell
BPR	=	bovine pancreatic ribonuclease
CD	=	cluster of differentiation
cDNA	=	complementary deoxyribonucleic acid
D	=	diversity (gene segment)
DNA	=	deoxyribonucleic acid
EAE	=	experimental allergic encephalomyelitis
ES	=	embryonic stem
FCS	=	fetal calf serum
HLA	=	human lymphocyte antigen
HPLC	=	high pressure liquid chromatography
Ig	=	immunoglobulin
J	=	joining (gene segment)
MHC	=	major histocompatibility complex
mRNA	=	messenger ribonucleic acid
MS	=	multiple sclerosis
NOD	=	nonobese diabetic (strain of mice)
PASA	=	polypeptide arthritogenic self antigen
PCR	=	polymerase chain reaction
RA	=	rheumatoid arthritis
RNA	=	ribonucleic acid
RNase	=	ribonuclease
ReA	=	reactive arthritis
REN	=	restriction endonuclease
TCR	=	T cell receptor
Tg	=	transgenic
tRNA	=	transfer ribonucleic acid
V	=	variable (gene segment)



## Glossary

*Affinity chromatography:* Chromatography in which the relatively immobilized substance has an affinity for a specific component of the mixture which is being chromatographed.

5       *Amino acid sequence:* The sequence of a polypeptide given in the order of from amino terminal (N-terminal), to carboxy terminal (C-terminal). Synonymous with "polypeptide sequence," "peptide sequence," "protein sequence," or "primary protein sequence."

10       *Animal:* (1) Excludes human beings, individually and collectively, in all stages of development, including embryonic and fetal stages, unless otherwise indicated; and (2) includes all other vertebrate animals, including an individual animal in any stage of development, including embryonic and fetal stages. "Non-human animal" has the same meaning as "animal."

15       *Animal model:* A non-human animal that faithfully mimics a human disease and in which potential therapeutic compositions or potentially hazardous compositions may be evaluated for their effect on the disease.

*Antibody:* A protein molecule synthesized by a B-cell upon exposure to antigen capable of combining specifically with that antigen. Synonymous with immunoglobulin (Ig).

20       *Antigen:* A molecule or composition of matter which (1) induces an immune response in an animal, and (2) interacts specifically with antigen-recognizing components of an immune animal's immune system.

25       *Self antigen:* A normal endogenous molecule or composition of matter in an animal which is an antigen in an autoimmune disease. Synonymous with autoantigen.

*Biomolecule:* A molecule produced by a living organism. Synonymous with "biological molecule."

*Biochemical synthesis:* The synthetic production of a biomolecule wherein other biomolecules are utilized in the production.

30       *Biosynthesis:* The production of a biomolecule by a living organism.

*Carrier:* A molecule required in combination with a hapten in order for an immune response to the hapten to occur. That is, a molecule which puts a hapten in a molecular context in which the hapten has enhanced immunogenicity.

5        *Chemical synthesis:* The synthetic production of a biomolecule in the absence of any other biomolecules and without requiring the use of any other biomolecules.

10       *Chromatography:* A process characterized by the uniform and uninterrupted flow of a mixture (gas or liquid) through a region of a stationary phase (liquid or solid) which, through various means, allows for the differential migration of the components of the mixture.

15       *Detectable label:* A chemical moiety that is coupled to a biomolecule to enable detection of the biomolecule and which may be selected from the group consisting of a radiolabel, an enzyme such as horseradish peroxidase or alkaline phosphatase, streptavidin, biotin, an epitope recognized by an antibody, and equivalents thereof.

*Detectably labelled:* A state of a biomolecule in which the biomolecule has covalently attached to it a detectable label.

20       *Disease:* (1) Excludes pregnancy *per se* but not autoimmune diseases associated with pregnancy, and (2) includes any abnormal condition of an organism or part, especially as a consequence of infection, inherent weakness, environmental stress, that impair normal physiological functioning.

*DNA sequence:* The sequence of contiguous nucleotide bases of a strand of DNA as read from 5' to 3'. Synonymous with "DNA molecule."

25       *Enzyme:* Protein that is a catalyst for a specific chemical reaction, often one involving one or more biomolecules as substrates and/or products. Unlike non-biologically derived catalysts, enzymes may recognize a substrate with stereospecificity, i.e., some enzymes are capable of recognizing, and thus catalyzing the chemical reaction of, only one of a pair of L- and D- enantiomers.

30

*Epitope:* A part of an antigen that interacts specifically with antigen-recognizing components of an animal's immune system. In a polypeptidic antigen, epitopes may correspond to short sequences of contiguous amino acids; the remainder of the antigen is called the *carrier*. Synonymous with antigenic determinant.

*Expression vector:* An artificial DNA sequence or a naturally-occurring DNA sequence that has been artificially modified, into which foreign or abnormal genes can be inserted for expression thereof in host organisms appropriate for the vector.

*Foreign or abnormal:* Non-endogenous to a healthy, wildtype animal. "Foreign or endogenous but abnormal antigens" designates biomolecules that an immune response should be generated towards and includes both biomolecules from invasive pathogens and animal-encoded biomolecules present on cancerous or infected cells; synonymous with "non-self." "Foreign or abnormal genes" designates DNA sequences that are not endogenous to an animal's genome, or animal-derived DNA sequences that have been rearranged, mutated, or otherwise genetically-engineered so as to possess properties (e.g., genomic location, regulation of expression, copy number) not possessed by the endogenous DNA sequences from which they were derived.

*Gene:* A DNA sequence that consists of a structural gene, e.g., a reading frame that encodes a polypeptide sequence, according to the standard genetic code (Table 2); and expression elements, e.g., promoters, terminators, enhancers, etc., required for transcription of the structural gene.

*Genetically engineered:* Subject to human manipulation intended to introduce genetic change.

*Hapten:* A small molecule which (1) cannot, by itself, induce an immune response in an animal, (2) can, in combination to a carrier to which it is bound, induce an immune response in an animal, and (3) interacts specifically with the antigen-recognizing components of an immune animal's immune system.

*Host animal:* An animal that harbors foreign and/or abnormal genes introduced as a result of (1) invasion of cells of the animal by a naturally occurring or genetically engineered intracellular parasite; or (2) introduction into cells of foreign or abnormal genes by human manipulation.

5       *Immune animal:* An animal which has been presented with an immunizing amount of antigen and has generated a humoral and/or cell-mediated immune response thereto.

*Isoschizomer:* One of a set of restriction enzymes that are functionally equivalent by virtue of each having the same recognition sequence.

10       *Mammal:* (1) Excludes human beings, individually and collectively, in all stages of development, including embryonic and fetal stages, unless otherwise indicated; and (2) includes all other animals that are members of the vertebrae class *Mammalia*, including an individual animal in any stage of development, including embryonic and fetal stages, distinguished by self-regulating body temperature, hair, and, in the females, milk-producing  
15       mammary glands.

*Monoclonal antibody:* A unique, isolated antibody molecule produced by a hybridoma.

20       *Monospecific antibody:* A polyclonal antibody produced in immunological response to a single or few epitopes found in (1) a short, isolated, synthetic antigen or (2) a short, isolated, carrier-bound hapten.

*Polyclonal antibody:* A composition that comprises an assortment of different antibodies that all recognize a particular antigen.

*Polypeptide:* A polymer of amino acid residues.

25       *Protein:* A biomolecule comprising one or more polypeptides arranged into a functional, three-dimensional form.

*Recombinant biosynthesis:* Synthesis in an organism that is directed towards a particular biomolecule by the natural introduction of genetic material encoding the biomolecule.

*Restriction endonuclease:* An endonuclease that cleaves DNA at each occurrence therein of a specific recognition sequence. Synonymous with "restriction enzyme."

5      *Transgene:* A gene that does not occur naturally in an animal, i.e., a foreign or abnormal gene, introduced into an animal by unnatural means, i.e., by human manipulation.

*Transgenic animal:* An animal into which has been introduced, by unnatural means, i.e., by human manipulation, one or more transgenes.

TABLE 2. THE GENETIC CODE

<i>First position (5' end)</i>	<i>Second position</i>				<i>Third position (3' end)</i>
5 U	U	C	A	G	U C A G
	Phe	Ser	Tyr	Cys	
	Phe	Ser	Tyr	Cys	
	Leu	Ser	Stop	Stop	
	Leu	Ser	Stop	Trp	
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Throughout the disclosure, abbreviations for amino acid and nucleotide residues present in, respectively, polypeptide and nucleic acid sequences, are as described in 37 C.F.R. § 1.822, revised as of July 1, 1993.

**TABLE 3. REN RECOGNITION SEQUENCES**

<b>REN</b>	<b>Recognition Sequence</b>
<b>BamHI</b> <b>EcoRI</b> <b>EcoRV</b>	<b>GGATCC</b> <b>GAATTC</b> <b>GATATC</b>
<b>KpnI</b> <b>NdeI</b> <b>NruI</b> <b>NotI</b>	<b>GGTACC</b> <b>CATATG</b> <b>TCGCGA</b> <b>GCGGCCGC</b>
<b>PstI</b> <b>SacII</b> <b>SalI</b> <b>XhoI</b> <b>XmaI</b>	<b>CTGCAG</b> <b>CCGCGG</b> <b>GTCGAC</b> <b>CTCGAG</b> <b>CCCGGG</b>

### *Detailed Description of the Disclosure*

The invention is based on the unexpected discovery that non-human animals having a substantially limited repertoire of T cell receptors display a phenotype selectively comprising severe classical symptoms of rheumatoid arthritis. The TCR repertoire of the arthritic animals, albeit limited, is functionally viable. The limited TCR repertoire of the arthritic animals consists in substantial part of TCRs that recognize one or more epitopes of endogenous polypeptide arthritogenic self antigens. In a preferred embodiment, the limited TCR repertoires of the arthritic animals result from the insertion into the genome of the animals of transgenes encoding TCR  $\alpha$  and  $\beta$  subunits that combine in T cells of the resultant transgenic animals to form a TCR that binds an antigen comprising one or more epitopes of an oligopeptide corresponding to amino acids 41-61 of bovine pancreatic ribonuclease (BPR) and having the amino acid sequence

Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-

Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1],

and/or one or more epitopes of a protein comprising endogenous polypeptide arthritogenic self antigens that are substantially cross-reactive with those of the BPR-derived oligopeptide.

The non-human animals of the invention comprise any animal having an arthritic phenotype as a result of transgenic expression of antigen-recognizing subunits of a T cell receptor that recognizes and/or binds one or more epitopes of amino acids 41-61 of bovine pancreatic ribonuclease and/or endogenous polypeptide arthritogenic self antigens (PASAs). Such non-human animals include vertebrates such as rodents, non-human primates, sheep, dog, cow, amphibians, reptiles, etc. Preferred non-human animals are selected from non-human mammalian species of animals, most preferably, animals from the rodent family including rats and mice, most preferably mice.



The transgenic animals of the invention are animals into which has been introduced by unnatural means (i.e., by human manipulation), one or more genes that do not occur naturally in the animal, e.g., foreign genes, genetically engineered endogenous genes, etc. The unnaturally introduced genes, known as transgenes, may be from the same or a different species as the animal but not naturally found in the animal in the configuration and/or at the chromosomal locus conferred by the transgene. Transgenes may comprise foreign DNA sequences, i.e., sequences not normally found in the genome of the host animal. Alternatively or additionally, transgenes may comprise endogenous DNA sequences that are abnormal in that they have been rearranged or mutated *in vitro* in order to alter the normal *in vivo* pattern of expression of the gene, or to alter or eliminate the biological activity of an endogenous gene product encoded by the gene. Watson, J.D., *et al.*, "The Introduction of Foreign Genes Into Mice," in *Recombinant DNA*, 2d Ed., W.H. Freeman & Co., New York (1992), pp. 255-272; Gordon, J.W., *Intl. Rev. Cytol.* 115:171-229 (1989); Jaenisch, R., *Science* 240:1468-1474 (1989); Rossant, J., *Neuron* 2:323-334 (1990).

The transgenic non-human animals of the invention are produced by introducing transgenes into the germline of the non-human animal. Embryonal target cells at various developmental stages are used to introduce the transgenes of the invention. Different methods are used depending on the stage of development of the embryonal target cell(s).

1. Microinjection of zygotes is the preferred method for incorporating transgenes into animal genomes in the course of practicing the invention. A zygote, a fertilized ovum that has not undergone pronuclei fusion or subsequent cell division, is the preferred target cell for microinjection of transgenic DNA sequences. The murine male pronucleus reaches a size of approximately 20 micrometers in diameter, a feature which allows for the reproducible injection of 1-2 picoliters of a solution containing transgenic DNA sequences. The use of a zygote for introduction of transgenes has the advantage that, in most cases, the injected transgenic DNA

sequences will be incorporated into the host animal's genome before the first cell division. Brinster, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:4438-4442 (1985). As a consequence, all cells of the resultant transgenic animals (founder animals) stably carry an incorporated transgene at a particular genetic locus, referred to as a transgenic allele. The transgenic allele demonstrates Mendelian inheritance: half of the offspring resulting from the cross of a transgenic animal with a non-transgenic animal will inherit the transgenic allele, in accordance with Mendel's rules of random assortment.

2. Viral integration can also be used to introduce the transgenes of the invention into an animal. The developing embryos are cultured in vitro to the developmental stage known as a blastocyte. At this time, the blastomeres may be infected with appropriate retroviruses. Jaenich, R., *Proc. Natl. Sci. (USA)* 73:1260-1264. Infection of the blastomeres is enhanced by enzymatic removal of the zona pellucida. Hogan, *et al.*, in *Manipulating the Mouse Embryo*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1986). Transgenes are introduced via viral vectors which are typically replication-defective but which remain competent for integration of viral-associated DNA sequences, including transgenic DNA sequences linked to such viral sequences, into the host animal's genome. Jahner, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:6927-6931 (1985); Van der Putten, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:6148-6152 (1985). Transfection is easily and efficiently obtained by culture of blastomeres on a mono-layer of cells producing the transgene-containing viral vector. Van der Putten, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:6148-6152 (1985); Stewart, *et al.*, *EMBO Journal* 6:383-388 (1987). Alternatively, infection may be performed at a later stage, such as a blastocoele. Jahner, D., *et al.*, *Nature* 298:623-628 (1982). In any event, most transgenic founder animals produced by viral integration will be mosaics for the transgenic allele; that is, the transgene is incorporated into only a subset of all the cells that form the transgenic founder animal. Moreover, multiple viral integration events may occur in a single founder animal, generating multiple transgenic alleles which will segregate in future

generations of offspring. Introduction of transgenes into germline cells by this method is possible but probably occurs at a low frequency. Jahner, D., *et al.*, *Nature* 298:623-628 (1982). However, once a transgene has been introduced into germline cells by this method, offspring may be produced in which the transgenic allele is present in all of the animal's cells, i.e., in both somatic and germline cells.

3. Embryonal stem (ES) cells can also serve as target cells for introduction of the transgenes of the invention into animals. ES cells are obtained from pre-implantation embryos that are cultured in vitro. Evans, M.J., *et al.*, *Nature* 292:154-156 (1981); Bradley, M. O., *et al.*, *Nature* 309:255-258 (1984); Gossler, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 83:9065-9069 (1986); Robertson *et al.*, *Nature* 322:445-448 (1986). ES cells that have been transformed with a transgene can be combined with an animal blastocyst, whereafter the ES cells colonize the embryo and contribute to the germline of the resulting animal, which is a chimera (composed of cells derived from two or more animals). Jaenisch, R., *Science* 240:1468-1474 (1988). Again, once a transgene has been introduced into germline cells by this method, offspring may be produced in which the transgenic allele is present in all of the animal's cells, i.e., in both somatic and germline cells.

Although the initial introduction of a transgene is a Lamarckian (non-Mendelian) event, the transgenes of the invention may be stably integrated into germ line cells and transmitted to offspring of the transgenic animal as Mendelian loci. Other transgenic techniques result in mosaic transgenic animals, in which some cells carry the transgenes and other cells do not. In mosaic transgenic animals in which germ line cells do not carry the transgenes, transmission of the transgenes to offspring does not occur. Nevertheless, mosaic transgenic animals are capable of demonstrating phenotypes associated with the transgenes.

Transgenes may be introduced into animals in order to provide animal models for human diseases. Transgenes that result in such animal models include, e.g., transgenes that encode mutant gene products associated with an

inborn error of metabolism in a human genetic disease and transgenes that encode a human factor required to confer susceptibility to a human pathogen (i.e., a bacterium, virus, or other pathogenic microorganism). Leder, P., *et al.*, U.S. Patent 5,175,383 (Dec. 29, 1992); Kindt, T.J., *et al.*, U.S. Patent 5,183,949 (Feb. 2, 1993); Small, J.A., *et al.*, *Cell* 46:13-18 (1986); Hooper, M., *et al.*, *Nature* 326:292-295 (1987); Stacey, A., *et al.*, *Nature* 332:131-136 (1988); Windle, J.J., *et al.*, *Nature* 343:665-669 (1990); Katz, J.D., *et al.*, *Cell* 74:1089-1100 (1993). Transgenic animals that are predisposed to a disease may be used to identify compositions that induce the disease and to evaluate the pathogenic potential of compositions known to induce the disease. Berns, A.J.M., U.S. Patent 5,174,986 (Dec. 29, 1992).

In the appropriate strain background, the transgenes of the invention confer a severe arthritic phenotype to transgenic animals as they develop. Although the transgenic genotype is reproducibly transferred to offspring in a Mendelian fashion, the ability of the transgenic phenotype (i.e., arthritis) to be fully manifested varies in different genetic backgrounds. In practicing the invention, it is desirable to maintain the transgenes of the invention in host animals that belong to a strain in which, due to the strain's particular genetic background, the transgenic genotype does not manifest its harmful arthritic phenotype to a detectable or significant degree. An transgenic animal line in which the transgene is maintained and inherited as a Mendelian locus, but in which the manifestation of a harmful transgenic phenotype is reduced or inhibited, is known as a transgenic maintenance line.

In the course of practicing the invention, animals of the transgenic maintenance line are crossed with animals having a genetic background in which the transgene fully manifests a deleterious arthritic phenotype. Offspring that have inherited the transgenes of the invention, and which will thus develop arthritis, are distinguished from littermates that have not inherited transgenes by analysis of genetic material from the offspring for the presence of biomolecules that comprise unique sequences corresponding to sequences of, or encoded by, the transgenes of the invention. For example,

biological fluids that contain polypeptides uniquely encoded by the transgenes of the invention may be immunoassayed for the presence of the polypeptides. A more simple and reliable means of identifying transgenic offspring comprises obtaining a tissue sample from an extremity of an animal, e.g., a  
5 tail, and analyzing the sample for the presence of nucleic acid sequences corresponding to the DNA sequence of a unique portion or portions of the transgenes of the invention. The presence of such nucleic acid sequences may be determined by, e.g., hybridization ("Southern") analysis with DNA sequences corresponding to unique portions of the transgene, analysis of the  
10 products of PCR reactions using DNA sequences in a sample as substrates and oligonucleotides derived from the transgene's DNA sequence, etc.

An animal's collection of diverse T cell receptors, each having  $\alpha$  and  $\beta$  with unique variable regions, is referred to as the TCR repertoire. In an individual animal, including a human, with a wildtype, non-suppressed  
15 immune system, the TCR repertoire confers to the immune system the ability to properly identify millions of different antigens. Molecular genetic techniques, such as PCR amplification and sequence determination of DNA molecules encoding the variable regions of functionally rearranged TCR genes, can be used to examine the TCR repertoire of an individual animal.  
20 Uematsu, Y., *Immunogenet.* 34:174-178 (1991). In like fashion, the TCR repertoire in a particular tissue or biological fluid can be examined. Oskenberg, J.R., *et al.*, *Proc. Natl. Acad. Sci. (USA)* 86:988-992 (1989); Oksenberg, J.R., *et al.*, *Nature* 345:344-346 (1990) and erratum, *Nature* 353:94 (1991); Uematsu, Y., *et al.*, *Proc. Natl. Acad. Sci. (USA)* 88:534-538  
25 (1991); Panzara, M.A., *et al.*, *Biotechniques* 12:728-735 (1992).

Introduction of the transgenes of the invention, which encode functionally rearranged TCR subunits, into an animal results in an animal in which the TCR repertoire consists substantially of the transgene expressed TCR. In transgenic TCR animals, endogenous genes for TCR subunits are not  
30 expressed due to the phenomena known as allelic exclusion. Alt, F.W., *et al.*, *Cell* 21:1-12 (1980); Early, P., and Hood, L., *Cell* 24:1-3 (1981). The

expression of a functionally rearranged TCR $\beta$  gene in transgenic mice prevents expression of endogenous TCR $\beta$  genes, apparently by inhibiting complete V-D-J joining. Weaver, D., *et al.*, *Cell* 42:117-127 (1985); Krimpenfort, P., *et al.*, *EMBO Journal* 7:745-750 (1988); Uematsu, Y., *et al.*, *Cell* 52:831-841 (1988). Transgenic expression of a functionally rearranged TCR $\beta$  chain that lacks a variable region suppresses endogenous TCR $\beta$  alleles and thus blocks normal T cell maturation. Krimpenfort, P., *et al.*, *Nature* 341:742-746 (1988); Krimpenfort, P.J.A., *et al.*, U.S. Patent 5,175,384 (Dec. 29, 1992). However, expression of a functionally rearranged TCR $\alpha$  chain does not always prevent rearrangement and expression of the endogenous TCR $\alpha$  genes. Bluthmann, H., *et al.*, *Nature* 334:156-159 (1988); Borgulya, P., *et al.*, *Cell* 69:529 (1992). Thus, transgenic expression in an animal of both a functionally rearranged TCR $\alpha$  gene, and a functionally rearranged TCR $\beta$  gene that comprises a variable region, results in a transgenic animal that has a functionally viable, albeit limited, TCR repertoire. Thus, in the transgenic animals of the invention, TCRs composed of the two transgene encoded TCR subunits will predominate over other types of TCRs.

The transgenes of the invention may be obtained by isolating or amplifying DNA sequences from genomic sources, or preparation of cDNA molecules from mRNA templates, by chemical or biochemical synthesis, or combinations thereof. The structural portions of the transgenes of the invention comprise exons, comprising portions of the reading frames for particular TCR $\alpha$  and TCR $\beta$  subunits, separated by non-TCR encoding introns. The introns are removed from mature mRNA molecules and amino acid sequences corresponding to the introns do not appear in proteins that result from biosynthesis directed by the mature mRNA sequences. Chambon, P., *Sci. American* 244:60-71 (1981). The structural portions of the transgenes of the invention encode TCR $\alpha$  and TCR $\beta$  subunits that comprise variable regions that compose, in the assembled TCR molecule, a variable region that binds an antigen comprising one or more epitopes of an oligopeptide corresponding to

amino acids 41-61 of bovine pancreatic ribonuclease (BPR) and having the amino acid sequence

Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-

Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1],

5 and/or one or more epitopes of proteins comprising endogenous polypeptide arthritogenic self antigens that are substantially cross-reactive with the epitopes of the BPR-derived oligopeptide.

In order to be expressed, the structural portions of the transgenes of the invention must be coupled to a promoter in a functional arrangement. The promoter need not be a naturally occurring promoter. Different types of promoters, directing different levels of expression, may be used to modulate the extent of arthritis in the transgenic animals of the invention. If a constitutive promoter is desired to be used, a viral promoter such as the SV40 early promoter, is preferred. Other promoters may be semi-constitutive or semi-inducible, i.e., having a basal level of expression that may be increased by administering to the transgenic animals particular inducing compositions. A truly inducible promoter, i.e., one that only functions when particular inducing compositions are present, may be used if stringent control of expression is desired. Additional promoter-associated regulatory elements may be include to enhance, decrease or temporally or histologically limit the expression of the transgenes of the invention. By these means, the biochemical, cellular, immunological and biological characteristics of the arthritis generated in the transgenic arthritic animals of the invention may be manipulated or controlled to produce useful variants thereof.

25 Immunologic methods by which the invention may also be practiced include immunization of the non-human animal with a polypeptide comprising amino acids 41-61 of bovine pancreatic ribonuclease, or an isolated protein comprising polypeptide arthritogenic self antigens (PASAs), or synthetic polypeptides having amino acid sequences derived from the amino acid

sequence of the isolated PASA-comprising protein of the invention. Alternatively, transfer of T cells from the transgenic arthritic animals of the invention into non-transgenic animal may be used to establish an autoimmune response therein. Arthritic animals produced by these immunologic methods, however, display the arthritic condition with a lesser degree of reproducibility and predictability of the course of the disease than the transgenic arthritic animals of the invention.

Other features and advantages of the invention will be apparent from the Examples and from the claims.

### *Examples*

The following Examples serve only to illustrate the invention, and are not to be construed as in any way limiting the invention.

#### *Example 1: Construction of Transgenes Encoding R28 TCR Subunits*

This example describes the production of isolated DNA sequences that encode the  $\alpha$  and  $\beta$  subunits of the T cell receptor displayed by R28 cells, and transgene constructs derived therefrom.

#### *DNA Sequences Encoding TCR Subunits from Hybridoma R28*

R28, also informally referred to as RNase 37, is a murine T cell hybridoma derived from a B10.A(4R) mouse injected with an oligopeptide corresponding to amino acids 41-61 of Bovine Pancreatic RNase (BPR). Peccoud, J., *et al.*, *EMBO Journal* 9:4215-4223 (1990). *In vitro*, the TCR displayed on R28 responds to the BPR-derived oligopeptide when it is presented by APCs displaying the MHC A<sup>k</sup> molecule. When APCs displaying a mutant MHC A<sup>k</sup> molecule are used, R28 cross-reactively recognizes an uncharacterized peptide antigen that is either present in fetal calf serum or the



synthesis of which is promoted by fetal calf serum, but which is not BPR or the BPR-derived oligopeptide. Dellabona, P., *et al.*, *Eur. J. Immunol.* 21:209-213 (1991).

5 DNAs sequences encoding many different V, D and J segments for Ig subunits are present in multiple unexpressed copies in germline DNA; a different but analogous collection of segments for TCR subunits is also present. For example, in the murine genome, the TCR $\beta$  subunit gene locus contains two tandemly arranged nearly identical C $\beta$  regions, each of which is preceded by one D and six J segments; the TCR $\beta$  locus also contains 20 to 30  
10 V segments. Davis, M.M., and Bjorkman, P.J., *Nature* 334:395-402 (1988). During development of an animal, diverse variable regions are generated in individual cells of the immune system by the random joining of different V, D and J (for TCR $\beta$ ), or V and J (for TCR $\alpha$ ) gene segments. At or about the same time, one or more nucleotides is inserted at the junction points of the  
15 gene segments, generating additional diversity in the gene segments encoding variable regions, particularly in the case of TCR variable regions. Sakano, H., *et al.*, *Nature* 280:288-294 (1979); Early, P., *et al.*, *Cell* 19:981-992 (1980); Davies, M.M., and Bjorkman, P.J., *Nature* 334:395-402 (1988).

The functionally rearranged variable and joining regions of both the  $\alpha$   
20 and  $\beta$  chains of the TCR were cloned and sequenced according to standard procedures. A functionally rearranged TCR or Ig subunit gene is one in which the DNA rearrangements have resulted in an expressed gene that encodes a functional TCR or Ig subunit, i.e., a subunit that is not prematurely terminated during biosynthesis because the reading frame has been altered, or  
25 a stop codon has been introduced into the reading frame, by the V-D-J joining events (large subunit) or V-J joining events (small subunit), and/or somatic mutation. Although the variable region of TCR subunits varies from T cell to T cell, the constant region remains the same. Thus, determination of the amino acid sequence of the variable regions of TCR $\alpha$  and TCR $\beta$  subunits, in  
30 combination with the known amino acid sequence of the constant regions of the subunits, defines a unique functional TCR molecule. Kappler, J., *et al.*,

*Cell* 35:295-302 (1983); Sim, G.K., *et al.*, *Nature* 312:771-775 (1984); Acuto, O., and Reinherz, E.L., *New England J. Med.* 312:1100-111 (1985); Yague, J., *et al.*, *Nucl. Acids Res.* 16:11355-11364 (1988).

5           Analysis of the DNA sequences encoding the variable regions of the subunits of TCR from R28 reveals that the TCR $\alpha$  variable sequence is V $\alpha$ 1-derived, and the TCR $\beta$  variable sequence is V $\beta$ 6-derived (Figure 1, Panels A and B). The unique amino acid sequences of the TCR $\alpha$  [SEQ ID No. 5] and TCR $\beta$  [SEQ ID No. 7] variable regions are useful for designing therapeutic compositions (see below). Equivalents to the DNA sequences encoding the  
10           amino acid sequence of the variable regions of R28 TCR subunits [SEQ ID Nos. 4 and 6] are produced by substituting codons that are equivalent, i.e., that encode the same amino acid residues as each other, according to the standard genetic code (Table 2).

### *Structure of the Transgenes*

15           Two DNA fragments, from plasmids paKRN and pbKRN, were used to generate the KRN transgenic line. Both plasmids contain DNA sequences initially derived from hybridoma R28. Peccoud, J., *et al.*, *EMBO Journal* 9:4215-4223 (1990). The functionally rearranged variable and joining regions of both the  $\alpha$  and  $\beta$  chains of the TCR were inserted into appropriate sites of  
20           cassette vectors which are designed so as allow for the proper insertion of functionally rearranged TCR variable regions into TCR subunit genes which are operably linked to murine expression sequences upon introduction into transgenic mice.

25           The structures of the resulting plasmids, paKRN and pbKRN, are shown in Figures 1A and 1B, respectively, along with the exact nucleotide sequences of the functionally rearranged gene fragments that encode the variable region of each TCR subunit. Each plasmid contains DNA sequences coding for a specific TCR subunit and signals required for the expression of the coding sequences in mice. The gene for the  $\alpha$  subunit is contained on

paKRN; pbKRN comprises the gene for the  $\beta$  subunit. Specifically, paKRN contains the TCR V $\alpha$  promoter region, an exon encoding the V $\alpha$ 4 variable region and joining segments isolated from R28, an intron which is absent in mature mRNA, an exon encoding the C $\alpha$  constant region, and 3' flanking DNA sequences including polyadenylation and other 3' expression signals. Plasmid pbKRN has basically the same structure, and contains the TCR V $\beta$  promoter region, an exon encoding the V $\beta$ 6 variable region and joining segments isolated from R28, an intron which is absent in mature mRNA, an exon encoding the C $\beta$  constant region, and 3' flanking sequences including polyadenylation and other 3' expression signals.

*Escherichia coli* cells containing either plasmid paKRN or pbKRN were deposited under the Budapest Treaty with the Collection Nationale de Culture de Micro-organismes (CNCM) of the Institut Pasteur, 28, Rue du Dr Roux, 75724 Paris Cédex 15, France, on May 18, 1994, and assigned the accession numbers I-1413 and I-1414, respectively. After issuance of a patent on this application, the CNCM will make the plasmids available to requestors in accordance with the Budapest Treaty and applicable U.S. patent laws and regulations. These deposits are not a license to practice the invention, and no admission is intended to be made that the deposits were necessary to satisfy the patent laws of the U.S. or any other country.

### ***Example 2: Construction of a Transgenic TCR Mouse Line***

This example describes the construction of transgenic mice that have TCR populations that consist substantially of the TCR $\alpha$  and TCR $\beta$  subunits encoded by the KRN transgenic allele. The KRN transgenic mice have a functionally viable, albeit limited, TCR repertoire.

### *Introduction of the Transgenes into the Murine Genome*

In order to generate DNA molecules suitable for use in microinjection, it was necessary to eliminate the DNA sequences of paKRN and pbKRN required solely for the maintenance of these plasmids in prokaryotic host cells. This was accomplished by treating the plasmids with the restriction endonuclease Sall (paKRN) or KpnI (pbKRN) and isolating the relevant Sall or KpnI restriction fragment, respectively (see Figure 1), by preparative agarose gel electrophoresis and electroelution. The restriction fragments were extracted twice with phenol-chloroform, precipitated with ethanol, and resuspended.

Isolated restriction fragments containing the TCR $\alpha$  and TCR $\beta$  transgenes were injected into B6xSJL F2 embryos according to standard procedures of transgenesis. Hogan, B., *et al.*, *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pages 1-332 (1986). Fertilized mouse eggs were recovered in cumulus from the oviducts of superovulated B6xSJL F1 females that had mated with males several hours earlier. The TCR $\alpha$  and TCR $\beta$  transgenes were injected in the most accessible pronucleus of each fertilized egg. Microinjected eggs were implanted into the oviducts of one-day pseudopregnant Swiss foster mothers and carried to term.

Several weeks after birth, a transgenic founder was identified by Southern hybridization with DNA molecules isolated from tail biopsies of the pups. The founder mouse possesses a genome which contains integrated copies of genes for both the TCR $\alpha$  and TCR $\beta$  subunits, and was crossed to C57Bl/6 mice to establish the KRN transgenic mouse line.

### *Transmission of Transgenes*

Mendelian transmission of a transgene is accomplished by crossing a transgenic mouse with a non-transgenic mouse. Typically, backcrosses are

with C57Bl/6 mice, but other strains are used. Transgene-positive offsprings are routinely identified by cytofluorimetric analysis of peripheral blood lymphocytes with reagents directed against the V $\beta$ 6 segment (see below), or by a nucleic acid hybridization assay. The hybridization assay includes the steps of obtaining a biological sample from each littermate, typically by physically separating the terminal 0.5 to 1.0 centimeters of the tail of the mouse, homogenizing or extracting the tail sample, purifying or amplifying DNA molecules therefrom, digesting the resultant DNA molecules with EcoRV restriction endonuclease, electrophoretically separating the restricted DNA sequences, transferring and binding the restricted, separated DNA sequences to a filter and hybridizing the filter-bound DNA sequences with a detectably labeled nucleic acid probe consisting of the approximately 0.7 kb XmaI-NotI fragment from paKRN (Figure 1A). Additionally or alternatively, the approximately 0.5 kb XhoI restriction fragment from pbKRN can be detectably labeled and used as a probe for transgenic offspring.

A representative example of a hybridization assay of a litter of KRN offspring is shown in Figure 2. Transmission of the transgene is Mendelian; half the offspring are positive for the transgene. The  $\alpha$  and  $\beta$  transgenes are closely linked on a mouse chromosome and appear to have co-integrated after micro-injection of the respective transgenic DNA sequences. The chromosomal locus comprising the TCR  $\alpha$  and  $\beta$  transgenes is referred to as the KRN transgenic allele.

#### ***Expression of the KRN Transgenic Allele***

Several lines of evidence indicate that both the  $\alpha$  and  $\beta$  transgenes are expressed in a large proportion of lymphocytes in the transgenic mice, and that T cell receptors composed of the transgenically encoded TCR subunits are the predominate members of a limited TCR repertoire in the transgenic animals.

*Expression of TCR $\alpha$ .* Expression of the TCR  $\alpha$  subunit encoded by the transgene was demonstrated by two methods.

First, TCR $\alpha$  transcripts from lymph node cells were amplified *en masse* via PCR reactions that use degenerate oligonucleotides in order to achieve random sampling of the TCR $\alpha$  repertoire. Candéais, S., *et al.*, *Proc. Natl. Acad. Sci. (USA)* 88:6167-6170 (1991). PCR products corresponding to amplified transcripts were cloned into an M13 bacteriophage vector, and plaques containing cDNA sequences corresponding to TCR $\alpha$  subunits were revealed by hybridization with a detectably labeled nucleic acid C $\alpha$ -specific probe comprising DNA sequences from the shared C $\alpha$  constant region, i.e.,

TTTGTTGGTATTGGAAGGGGCCAGAGCACAGAAGTACACG [SEQ ID No. 8].

Among TCR $\alpha$  cDNA clones, clones corresponding to transgene-encoded transcripts were detected by hybridization with a detectably labeled nucleic acid transgene-specific probe, consisting of the approximately 0.7 kb XmaI-NotI fragment from paKRN (Figure 1A) and corresponding to the functionally rearranged V-J joining region contained in paKRN, which specifically hybridizes to cDNA sequences derived from the transgene encoded TCR $\alpha$  variable region. Of the clones that were detected by hybridization with the C $\alpha$ -specific probe, 97% were also detected by hybridization with the transgene-specific probe. This result indicates that mRNA sequences corresponding to the transgene encoded TCR $\alpha$  subunit account for the vast majority of TCR $\alpha$  transcripts in the transgenic animals.

Second, an anti-idiotypic serum was raised in normal mice by immunization with transgenic lymph node cells. The anti-idiotypic serum contains polyclonal antibodies that recognize an epitope found in or near the variable region of the predominant TCRs from KRN transgenic mice.

The specificity of the anti-idiotypic serum for the TCR of hybridoma R28 was verified by demonstrating that it specifically recognized and stained T hybridomas and transfected cells expressing the TCR  $\alpha$  and  $\beta$  chains from R28, or other T cells, e.g., KLy 11.10, Tris and BDC2.5. CD4<sup>+</sup>

lymphocytes were isolated from wildtype and KRN transgenic mice according to standard methods. Wysocki, L.J., and Sato, V.L., *Proc. Natl. Acad. Sci. (USA)* 75:2844-2848 (1978); Wasik, M.A., and Morimoto, C., *J. Immunol.* 144:3334-3340 (1990); Harriman, G.R., *et al.*, *J. Immunol.* 145:2035-2043 (1990); Koulouva, L., *et al.*, *J. Immunol.* 145:2035-2043 (1990). CD4<sup>+</sup> lymphocytes were incubated with a saturating dose of anti-idiotypic sera at 4°C for 15 minutes, washed, and incubated with detectably labeled (i.e., fluorescently tagged by conjugation to FITC) goat anti-mouse antibodies for 45 minutes at 4°C. After washing, the cells were fixed with 1% formaldehyde and analyzed by passage through a fluorescence-activated automatic cell sorter (e.g., a Becton-Dickinson FACScan®). Kung, P.C., and Goldstein, G., U.S. Patents 4,381,295 (Apr. 26, 1983), 4,364,932 and 4,364,936 (both Dec. 21, 1982). The cytofluorimetric analysis indicates that the anti-idiotypic serum reacts with a majority of CD4<sup>+</sup> lymphocytes in transgenic mice, but does not react with CD4<sup>+</sup> lymphocytes from control animals with only low intensity (Figure 3, Panel A).

**Expression of TCR $\beta$ .** Expression of TCR $\beta$  chain transgene in KRN mice was verified by cytofluorimetric analysis of CD4<sup>+</sup> lymphocytes. The RR4.7 monoclonal antibody (commercially available from Pharmingen, San Diego, CA; catalog No. 01361C), which recognizes the amino acid sequence encoded by the V gene segment (V $\beta$ 6) present in the functionally rearranged TCR $\beta$  of the transgene, was used. As indicated by reaction with the RR4.7 monoclonal antibody, V $\beta$ 6 was present on essentially all CD4<sup>+</sup> lymphocytes in transgenic mice (Figure 3, Panel B). This assay can be used to identify transgene-positive animals in mixed litters.

The antigen specificity imparted by TCRs composed of the  $\alpha$  and  $\beta$  chains of the R28 TCR could be detected in peripheral lymph node cells. Strong proliferation of cells was detected when lymph node T cells were incubated in the presence of the BPR-derived oligopeptide having the amino acid sequence

Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-  
Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1]

and APCs expressing MHC molecules.

*Example 3: Isolation and Characterization of Transgenic Arthritic Mice*

5           The new transgenic model of arthritis describe herein did not result  
from a directed effort towards this goal. The KRN line of transgenic mice  
was constructed by introducing into mice the genes encoding the  $\alpha$  and  $\beta$   
subunits of the T cell receptor for antigen (TCR) from a murine T cell  
10       hybridoma reactive to bovine pancreatic RNAase, in order to address  
questions of fundamental immunology pertaining to the selection of the T cell  
repertoire. In the genetic background of the strain of mice into which the  
KRN transgene was initially introduced, C57Bl/6, the transgene did not confer  
an abnormal phenotype. Of course, due to their limited TCR repertoires,  
these mice may have a reduced ability to mount appropriate immune responses  
15       against pathogens that are, in any event, less frequently encountered in the  
laboratory environment.

          The C57Bl/6 KRN-transgenic mice were then adventitiously crossed  
with inbred mice of the NOD strain. Surprisingly, all of the transgene-  
positive offspring from this cross develop severe symptoms associated with  
20       RA in humans, i.e., chronic inflammation and deformation of the limb joints.  
There is a 100% penetrance of the disease in NOD KRN-transgenic animals,  
i.e., every NOD mouse that inherits the KRN transgenic allele develops  
arthritis, as shown in Figure 5. Males are affected as well as females. There  
is no arthritis when the transgenic mice are not of the NOD background  
25       (Figure 5, KRNxB6), and such mice are used as transgenic maintenance  
animals.



### *Clinical Course of Arthritis*

The progression of arthritis in mice carrying the KRN transgene and NOD-derived MHC genes required for penetrance of the arthritic genotype (see below) was followed by calliper measurements of the thickness of the ankle joints of arthritic animals, a simple assay which gives measurements that correlate well with the onset and evolution of the inflammatory process. Transgenic arthritic mice show signs of joint inflammation, with a sharp onset between three and four weeks of age; no clinical signs are apparent before that time (Figure 4B). The distal joints appear more affected than the knee or elbow. The joints are swollen and red; deformation sets in after a few weeks, with the expected consequence that limb motility is reduced (see Figure 4A). This state persists for several weeks. After ten to twelve weeks, the inflammatory symptoms tend to subside somewhat, but major sequellar deformations persist.

Because of the serious reduction in motility that their arthritis imparts, NOD KRN-transgenic mice are poor breeders. However, litters from NOD KRN-transgenic parents are obtainable. In practice, arthritic animals are generated by maintaining the KRN transgene in mice of the C57Bl/6 background, wherein arthritic symptoms are not manifested, and crossing transgene positive mice with NOD mice. All KRN transgene-positive offspring animals which result from this cross develop arthritis and are identified prior to the onset of symptoms by nucleic acid hybridization or cytofluorimetric analysis.

### *Radiography*

Radiography (x-ray imaging) was performed on individual NOD KRN-transgenic animals as well as non-transgenic littermates at various times after disease onset in the transgenic animals. Bone and cartilage erosion is clearly visible in the knee and ankle (Figure 6), reflecting the gross deformations that

are externally visible (compare with Panel A of Figure 4). Osteosynthesis with osteophytosis is widespread. In older animals, there is considerable rearrangement of the joints, consistent with the deformations visible externally. The spinal column also appears somewhat affected in radiographs.

5      ***Histopathology***

         Joints from normal or arthritic (KRN-transgenic NOD) mice were examined by histological examination of decalcified paraffin sections of spine, knee or paw joints, after staining with hematoxylin-eosin according to standard and routine pathology techniques. Stevens, A., in *Theory and Practice of*  
10      *Histological Techniques*, Bancroft, J.D., and Stevens, A., eds., 3rd Ed., Churchill Livingstone, Edinburgh, New York, pp. 107-118 (1990). There are very obvious signs of joint inflammation in the transgenic arthritic mice that parallel developments in patients with RA: synovial hyperplasia with several superimposed layers of large synoviocytes, polymorphic cellular infiltration  
15      (granulocytes, lymphocytes, plasmocytes), fibrinous exudate in the joints. Cartilage erosion, destruction and resorption is prevalent after a few weeks of disease. Again, lesions are predominant in the distal joints, clear but less active in the knee, and very discrete in the hip. The spine is also affected by inflammatory infiltrates, but mildly. No histological lesions are present before  
20      three weeks of age.

         It is important to note that signs of inflammation or infiltration have not been detected in any other organ examined. In sum, the autoimmune response in the transgenic arthritic mice is severe but, as in RA, is limited to synovial tissues, particularly cartilage. Thus, the transgenic arthritic animal  
25      model of the invention, unlike previously described animal models of RA, faithfully reproduces the distinguishing features of human RA.

### ***Requirements for the KRN Phenotype***

The initial observation of transgenic arthritis was made in F1 mice, originating from a cross between a mouse carrying the KRN transgenic allele in the C57Bl/6 background and an inbred NOD/Lt mouse. The resultant KRN-transgenic NOD animals have mixed alleles at each genetic locus in their genomes, consisting of an equal contribution of alleles from both the NOD/Lt parent and the C57Bl/6 parent. Since the KRN transgenic allele did not give rise to any arthritic symptoms when present in the C57Bl/6 background, genetic elements (i.e., one or more alleles) derived from the NOD genome contribute to the induction of arthritis by the KRN transgenic allele.

In order to identify the required NOD allele(s), non-NOD KRN-transgenic mice were crossed with mice of the B6.NH-2<sup>nod</sup> strain (gift of Dr. H. Kikutani). The B6.NH-2<sup>nod</sup> strain is a chimeric strain derived from the NOD and B6 strains of mice. In B6.NH-2<sup>nod</sup> mice, all genetic loci have the same alleles as B6, except for the MHC locus, which is derived from the NOD strain of mice. Arthritic disease was manifested in the progeny from the cross of non-NOD KRN-transgenic and B6.NH-2<sup>nod</sup> mice, with the same prevalence and characteristics as in a control cross of KRN-transgenic and NOD mice (Figure 5, KRNxB6.NH-2<sup>nod</sup> and KRNxNOD, respectively). In contrast, offspring of a cross between KRN-transgenic mice and B6 mice were not arthritic (Figure 5, KRNxB6), and there was no arthritic disease in other genetic combinations tested (i.e., in crosses of KRN-transgenic mice from B10.D2, B10.M, B10.S, B10.BR, B10.A, and B10.A(4R) strains).

Thus, arthritic disease resulting from the KRN transgenic allele is fully manifested due to genetic elements provided by particular MHC loci of different strains of mice. In particular, the MHC locus of the NOD strain of mice results in full manifestation of the KRN phenotype. MHC loci from other strains of mice, or MHC loci from other animals including humans, are testable in the mouse model for their ability to enable the KRN phenotype (see below). Thus, the invention provides a method for identifying and isolating

additional, non-NOD derived MHC loci that can be used to practice the invention.

### *Immunology of the Transgenic Arthritic Mice*

5 The very nature of the transgenic allele which directs arthritis in KRN-transgenic NOD mice implies a determining role of T lymphocytes in the disease and, by extension by analogy, in RA. Indeed, extensive clonal deletion of the central and peripheral T cell populations of the transgenic arthritic mice occurs during development of the animals. The mechanism of clonal deletion is very active around birth; few, if any, CD4<sup>+</sup> and CD8<sup>+</sup> T  
10 cells are present in the peripheral lymphoid organs at that time. The depletion of T cells tends to abate somewhat with age, and CD4<sup>+</sup> lymphocytes appear in animals of about three weeks of age in the spleen and lymph nodes.

The B cell component of the immune system of transgenic arthritic mice is less affected, with relatively normal numbers and distribution of B  
15 cells. However, there is an imbalance in the isotype of circulating IgG, with a marked increase of the IgG1 isotype in KRN-transgenic NOD mice. Rheumatoid factors (i.e., antibodies to the constant region of IgG antibodies) have not been detected in the transgenic arthritic mice. However, after two or three months, the spleen and lymph nodes of many KRN-transgenic NOD  
20 mice are grossly enlarged, presumably in response to the constant inflammatory state of the animal.

### *Example 4: Methods of Evaluating Anti-Arthritic Compositions*

Because of the reliable and reproducible nature of arthritis in the transgenic arthritic mice of the invention, and its clear relationship to human  
25 rheumatoid arthritis, the transgenic animals of the invention are used to estimate the safety and efficacy of anti-arthritis compositions under development. Anti-arthritic compositions are administered prior to the onset

of clinical manifestations, or after the disease has set in but before irremediable joint destruction and deformation have taken place.

### ***Therapeutic Immunointervention***

5 KRN-transgenic NOD arthritic mice were treated with anti-CD4 antibody, a composition that has shown some promise in alleviating the symptoms of RA in humans. Moreland, L.W., *et al.*, *Arthritis Rheum.* 36:307-319 (1993). The progress of arthritis in the treated animals, and in untreated control animals, was monitored by clinically examining the mice every third day. As shown in Figure 7, treatment of transgenic arthritic mice  
10 with anti-CD4 antibody from two weeks of age prevented the appearance of pathological manifestations.

The animal model of the invention is used to evaluate other potential anti-arthritic compositions, including but not limited to glucocorticoids, lymphokines, interferons, conjugates of cytotoxins with antibodies, CD  
15 proteins, MHC proteins, derivatives of CD and MHC proteins, and synthetic compounds or isolated effector biomolecules that influence antigen presentation by MHC molecules, TCR recognition of MHC-presented antigens, and/or interactions between MHC molecules and CD proteins. Because the transgenic arthritic animals of the invention develop arthritic  
20 symptoms in a predictable fashion, various effects of anti-arthritic compositions tested in the animal model of the invention can be examined in more rigorous fashion than was previously possible.

Anti-arthritic compositions can achieve their effect by preventing inflammation, by limiting the inflammatory response, or by limiting the  
25 destruction of tissue that occurs subsequent to inflammation. Administering an anti-arthritic composition to the transgenic animals of the invention at defined postnatal timepoints reveals if the composition exerts its effect at an early or late stage of arthritis. For example, anti-arthritic compositions that prevent inflammation will not be effective if administered after the

inflammatory response has initiated, unlike anti-arthritic compositions that prevent sequellar destruction of tissue.

5 The potency of anti-arthritic compositions is evaluated in terms of causing a statistically significant delay in the time of onset and/or the extent of inflammation and/or irremediable joint destruction and limb deformation in the transgenic arthritic animals of the invention. Changes in cellular immunological parameters of the transgenic arthritic animals of the invention, e.g., the number and distribution of CD4<sup>+</sup> or CD8<sup>+</sup> cells or of particular TCR or Ig molecules, in response to anti-arthritic compositions are determined to  
10 elucidate underlying mechanisms of therapeutic immunointervention.

### *Genetic Therapy*

Transgenes comprising DNA sequences encoding potentially anti-arthritic gene products, including polypeptides, are introduced into the genome of a mouse. Candidate anti-arthritic transgenes are introduced into the  
15 transgenic arthritic animal model of the invention by mating an offspring of the anti-arthritic transgenic founder animal with transgenic arthritic mice treated with anti-CD4, or another anti-arthritic agent, thereby imparting the transgenic arthritic mice sufficient mobility to undertake the mating process with vigor. Alternatively, candidate anti-arthritic transgenes are introduced  
20 into the genomes of individuals from the NOD strain of mice, and an offspring of the NOD founder is mated with a non-arthritic KRN-transgenic mouse and the development of arthritic symptoms in singly- and doubly-transgenic offspring is monitored and compared. An anti-arthritic DNA sequence is one which, when expressed in a transgenic arthritic animal that  
25 develops arthritis in the absence of the anti-arthritic DNA sequence, results in reduced, delayed or no inflammation or which limits or eliminates sequellar destruction of tissue subsequent to inflammation.

### ***Example 5: Methods of Evaluating Arthritogenic Compositions***

In KRN transgenic mice with an MHC locus from the NOD strain of mice, all transgenic mice develop arthritis. KRN transgenic mice with an MHC locus from strains of mice in which the KRN transgenic allele fails to induce arthritis are used to evaluate compositions for their arthritogenic potential. Compositions which enhance the arthritic potential of the KRN transgenic allele are arthritogenic in the animal model of the invention. Such compositions include but are not limited to glucocorticoids, lymphokines, interferons, conjugates of cytotoxins with antibodies, CD proteins, MHC proteins, derivatives of CD and MHC proteins, and synthetic compounds or isolated effector biomolecules that influence antigen presentation by MHC molecules, TCR recognition of MHC-presented antigens, and/or interactions between MHC molecules and CD proteins. Isolated effector biomolecules (e.g.,  $\gamma$ -interferon) that may be more prevalent in human females, and which have thus been suggested to be related to the increased incidence of RA therein (Fox, H.S., *et al.*, *J. Immunol.* 146:4362-4367 (1991)), are tested in both male and female transgenic non-human animals of the invention in order to eliminate effects due to other gender differences.

Arthritogenic compositions also include transgenes encoding an MHC protein, wherein DNA sequences expressed by the transgenes are derived from the MHC loci of animals including humans. Some MHC proteins influence the occurrence of autoimmune disease, and specific variant MHC alleles have been demonstrated to be correlated with a higher risk of certain human autoimmune diseases. In humans, wherein MHC antigens are known as HLA (human lymphocyte antigen) proteins, for example, individuals with the HLA-DR4 allele are six times more likely than those with other HLA variants to acquire rheumatoid arthritis. Steinman, L., *Sci. American* 269:106-114 (1993). Yersinia arthritis and ankylosing spondylitis are highly correlated with the HLA-B27 allele. Vaughan, J.H., in *Immunological Diseases*, Vol.

II, 3rd Ed., Samter, M., ed., Little, Brown and Company, pages 1029-1037 (1978).

As demonstrated by experiments with transgenic mice, the mouse T cell repertoire is perfectly capable of using human MHC gene products (HLA proteins). Kievits, F., *et al.*, *Nature* 329:447-449 (1987). DNA sequences encoding particular HLA molecules from humans are isolated and genetically engineered so as to be capable of introduction into the mouse genome and expression in murine cells. Utilization of human MHC gene products in mice requires the concomitant presence of  $\beta_2$ -microglobulin protein molecules; accordingly, DNA sequences encoding human  $\beta_2$ -microglobulin are similarly genetically engineered. Kievits, F., *et al.*, *Nature* 329:447-449 (1987). The transgenes are introduced into the genome of a mouse, and an offspring of a doubly transgenic (MHC and  $\beta_2$ -microglobulin) founder is mated with a non-arthritic KRN-transgenic mouse. Doubly-transgenic (MHC and  $\beta_2$ -microglobulin) and triply-transgenic (MHC,  $\beta_2$ -microglobulin and KRN) offspring are identified via the hybridization assay described above, using probes derived from the DNA sequences of the MHC,  $\beta_2$ -microglobulin and KRN transgenes. The occurrence and development of arthritic symptoms in triply-transgenic offspring is monitored and compared to that of doubly- and non-transgenic littermates.

An arthritogenic DNA sequence is one which, when expressed in a transgenic arthritic animal that does not develop arthritis in the absence of the arthritogenic DNA sequence, results in partial or full manifestation of the KRN phenotype. DNA sequences that are testable by the method of the invention include but are not limited to those of human HLA-B27, HLA-DR4 and other genes encoding human MHC proteins (in conjunction with transgenes encoding human  $\beta_2$ -microglobulin) (Kievits, F., *et al.*, *Nature* 329:447-449 (1987)), and MHC alleles from animals for which guarantees of prolonged mobility are sought (e.g., racehorses) in conjunction with  $\beta_2$ -microglobulin genes appropriate for the species of animal contributing the MHC allele to be tested. The animal model of the invention provides a basis



to determine underlying cellular and molecular mechanisms of observed correlations between particular MHC alleles and various forms of human arthritis. For example, the human MHC protein encoded by HLA-B27 is associated with some types of arthritis in humans (Vaughns, *supra*) and, when transgenically expressed in mice, is able to display antigens to murine TCRs (Kievits *et al.*, *supra*). This approach is more satisfying, at least in the mechanistic sense, than determining correlations between particular human MHC alleles and arthritic diseases.

The potency of arthritogenic compositions is evaluated in terms of causing a statistically significant advancement in the time of onset of arthritis and/or increase in the extent of inflammation and/or irremediable joint destruction and limb deformation in the transgenic arthritic animals of the invention. Alternatively or additionally, the potency of arthritogenic compositions is evaluated in terms of a statistically significant increase in the incidence of arthritis or arthritic symptoms in the KRN-transgenic non-arthritic animals of the invention. Changes in cellular immunological parameters of the transgenic animals of the invention, e.g., the number and distribution of CD4<sup>+</sup> or CD8<sup>+</sup> cells or of particular TCR or Ig molecules, in response to arthritogenic compositions are determined to elucidate underlying mechanisms of therapeutic immunointervention.

***Example 6: Isolation of Immune System Components that Bind  
Endogenous Polypeptidic Arthritogenic Self Antigens***

This example describes methods of isolating polypeptide antigens that are recognized by transgene encoded TCRs in the transgenic arthritic mice of the invention. These polypeptides are used to isolate cells and effector biomolecules from the immune systems of the transgenic arthritic animals of the invention that specifically bind one or more epitopes of murine proteins comprising polypeptide arthritogenic self antigens (PASAs). The isolated cells

and effector biomolecules are used to isolate PASA-comprising proteins from other species of animals including humans (Example 7).

*Oligopeptides Presented by APCs in Arthritic Animals.* A prerequisite for the subsequent examples is the identification of endogenous polypeptide antigens that are presented to the transgene encoded TCR of the invention during the etiology of arthritis. To this end, antigen presenting cells (APCs) are isolated from transgenic arthritic mice at various times before and during the development of arthritic symptoms. Of particular interest are APCs present in the thymus during the active period of clonal deletion around birth, however, APCs are also isolated from other tissues and at other times during the life of the mice. Heparinized venous blood is obtained from the transgenic arthritic mice of the invention, and polymorphonuclear blood cells (PMBCs) are isolated therefrom by, e.g., Ficoll-Hyapque density gradient centrifugation. Preparations of PMBC are depleted of monocytes and/or macrophages by plastic adherence for 2 hours at 37°C in tissue-culture flasks. Adherent cells are recovered by scraping, resuspended in an appropriate buffered media which does not include fetal calf serum (FCS) as fetal calf serum contains antigen(s) which can be recognized by the TCR of R28. Dellabona, P., *et al.*, *Eur. J. Immunol.* 21:209-213 (1991). The resuspended cells are used a source of APCs. Koulova, L., *et al.*, *J. Immunol.* 145:2035-2043 (1990).

APCs are treated with 0.025% trypsin to enhance the harvest of oligopeptides. Oligopeptides associated with MHC molecules are isolated according to the method of Slingluff, C.L., *et al.*, *J. Immunol.* 150:2955-2963 (1993), and fractionated by reversed phase high pressure liquid chromatography (HPLC), although other separation techniques may be used. Lehninger, A.L., *Biochemistry*, 2d Ed., pp.95-121. HPLC fractions containing relevant peptides are identified by activation of R28 hybridoma cells, as measured by the production of interleukin-2, in the presence of APCs isolated from non-arthritic NOD mice. Miedema, F., and Melief, C.J.M.,

*Immunol. Today* 6:258-259 (1985). HPLC fractions containing activity are pooled and rechromatographed as required in order to generate fractions of sufficient purity for subsequent uses intended. However, even relatively crude fractions suffice to identify hybridomas producing effector biomolecules specific for endogenous polypeptide arthritogenic self antigens (PASAs).

If sufficient homogenous material is generated, then the oligopeptides of R28-stimulatory HPLC fractions are sequenced by standard methods, including but not limited to repetitive Edman degradation. Lehninger, A.L., *Biochemistry*, 2d Ed., pp.95-121 (1978). Alternatively, tandem mass spectrometry (TMS) is used to identify the sequences of the most abundant peptides. Cox, A.L., *et al.*, *Science* 264:716-719 (1994). Although TMS cannot distinguish between Ile and Leu amino acid residues in a polypeptide, TMS generates amino acid sequence data that can be used to design pairs of oligopeptides alternately comprising Ile or Leu. Oligopeptides comprising TMS-derived amino acid sequences are chemically synthesized according to standard methods (Stewart and Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockland, Illinois (1985)) and purified from reaction mixtures by reversed phase HPLC. Isolated synthetic oligopeptides are tested for their ability to stimulate the R28 hybridoma in the presence of APCs isolated from non-transgenic NOD mice, and in this manner the sequences of actual R28-stimulatory oligopeptides are determined. These amino acid sequences correspond to short polypeptide fragments derived from endogenous PASA-comprising proteins.

### *Production of Hybridomas*

The spleen and thymus are isolated post-mortem from an arthritic transgenic mouse (e.g., a KRN-transgenic NOD mouse). Lymphocytes (T or B cells) are prepared from the isolated spleens or thymus of one or more transgenic animals at various times during the development of arthritis. Individual antibody-producing T or B cells are fused *in vitro* with

immortalizing cells. The resulting stably reproducing fused cell lines (hybridomas), each of which expresses TCRs (T cells) or antibodies (B cells) with a unique antigen-binding (variable) regions, are cultured by standard lymphocyte culturing techniques. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., pages 139-281 (1988); Ausubel, F.M., *et al.*, Eds., *Current Protocols in Molecular Biology*, Vol. 2, Greene Publishing Associates and John Wiley & Sons, pages 11.4.1-11.11.5 (1993).

T cell hybridomas are screened for activation (measured as interleukin-2 secretion) by APCs prepared from NOD mice in the presence or absence of an oligopeptide corresponding to amino acids 41-61 of bovine pancreatic ribonuclease (BPR) and having the amino acid sequence

Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-

Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1].

Alternatively, the T cell hybridomas are screened for activation in the presence of the BPR-derived oligopeptide and APCs displaying a wildtype or mutant A<sup>k</sup> MHC molecule due to transfection with a regulatable A<sup>k</sup> MHC gene. Dellabona, P., *et al.*, *Eur. J. Immunol.* 21:209-213 (1991). Either type of APC is alternatively used with crude or synthetic oligopeptides derived from PASA-comprising proteins (see above). In any event, R28 hybridoma cells are used as positive controls. The T cell hybridomas identified and isolated in this manner express TCR subunits which are either identical to those encoded by the transgenes of the invention, or mutants thereof produced in the transgenic arthritic mice and having altered binding characteristics.

B cell hybridomas are screened for the production of a monoclonal antibody that recognizes the BPR-derived oligopeptide and/or crude or synthetic oligopeptides derived from PASA-comprising proteins (see above). The desired B cell hybridomas are identified by ELISA assays using the BPR-derived oligopeptide and/or crude or synthetic oligopeptides derived from

PASA-comprising proteins as antigen. Monoclonal antibodies produced by the identified hybridomas are purified if necessary. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., pages 139-281 (1988); Ausubel, F.M., *et al.*, Eds., *Current Protocols in Molecular Biology*, Vol. 2, Greene Publishing Associates and John Wiley & Sons, pages 11.4.1-11.11.5 (1993).

### ***Monospecific Antibodies***

The crude or synthetic oligopeptides derived from endogenous PASA-comprising proteins (see above) are used to generate monospecific antibodies that are used in subsequent examples.

A "polyclonal antibody" refers to a composition that comprises an assortment of different antibodies that all recognize a particular antigen. Although useful in a variety of ways, polyclonal antibodies suffer from limitations due to their heterologous composition. For example, cross-reactivities of some polyclonal antibodies to antigens other than the one used to induce an immune response are commonly observed. The specificity of a polyclonal antibody can be limited to a particular epitope by using an isolated or synthetic antigen or hapten corresponding to a single chosen epitope. The antigen, or the hapten in association with a carrier, is used to generate a humoral immune response in an animal. The polyclonal antibody that results is specific for the chosen epitope and is referred to as a "monospecific antibody" or "antipeptide antibody." In particular, synthetic oligopeptides are used as antigens or haptens to generate monospecific antibodies that can be used to assay or isolate a protein comprising a sequence that corresponds with, or is similar to, the sequence of the oligopeptide.

The crude or synthetic oligopeptides derived from endogenous PASA-comprising proteins (see above) are used to generate monospecific antibodies according to standard methods. Wilkins, T.D., *et al.*, U.S. Patent 4,879,218 (Nov. 7, 1989); Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*,

Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., pages 53-137 (1988); Ausubel, F.M., *et al.*, Eds., *Current Protocols in Molecular Biology*, Vol. 2, Greene Publishing Associates and John Wiley & Sons, pages 11.12.1-11.15.4 (1993).

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***Example 7: Isolation of Proteins Comprising Polypeptide  
Arthritogenic Self Antigens (PASAs)***

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During development of the transgenic arthritic mice of the invention, an initiating immunologic event triggers a cascade of immunologic events that culminates in the transgenic animals developing severe arthritic symptoms in a reproducible and thus predictable manner. Because the KRN transgenic allele results in a majority of T cells in an individual mouse having a specific TCR, and because antigen-presenting MHC proteins from particular strains of mice are required for the transgenic phenotype to be manifested, it is possible that an important immunologic event that occurs relatively early in arthritis is recognition of endogenous self antigens by T cells. These endogenous self antigens are generally referred to herein as endogenous polypeptide arthritogenic self antigens (PASAs). The murine protein comprising these PASAs is believed to correspond to a human protein of primordial importance in the genesis of human RA. Human PASA-comprising proteins, and synthetic and derivatized compounds derived from human PASA-comprising proteins, are useful in the diagnosis, prevention or therapy of arthritis in humans (see subsequent examples). The transgenic arthritic animals of the present invention provide the means by which PASA-comprising proteins, or DNA sequences encoding such proteins, may be isolated from many animal sources including humans.

### ***Purification of PASA-Comprising Proteins by Immunodetection***

The monoclonal or monospecific antibodies of the previous example are used as probes in known immunohistological techniques in order to identify the physical distribution of PASA-comprising proteins in normal and arthritic mice, and in larger animals (e.g., sheep, dogs, cows, horses, etc.) from which sufficient material for protein purification is more readily obtained, according to standard methods. If necessary, animals at various stages of development are so examined. Ausubel, F.M., *et al.*, Eds., *Current Protocols in Molecular Biology*, Vol. 2, Greene Publishing Associates and John Wiley & Sons, pages 14.0.1-14.6.13 (1993).

Tissues having relatively high concentrations of PASA-comprising proteins are removed from animals post-mortem and used as a source of PASA-comprising proteins for purification thereof. As a purification assay, the activation of R28 cells, described in a previous example, is used. Standard methods of protein purification are used. Lehninger, A.L., *Biochemistry*, 2d Ed., pp.95-121. Additionally or alternatively, affinity columns comprising as binding agents the monoclonal or monospecific antibodies of the previous example are used. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., pages 511-552 (1988). The purified PASA-comprising proteins are sequenced by standard methods, including but not limited to partial proteolysis and repetitive Edman degradation. Lehninger, A.L., *Biochemistry*, 2d Ed., pp.95-121 (1978).

### ***DNA Sequences Encoding PASA-Comprising Proteins***

The amino acid sequence of PASA-derived oligopeptides bound to APCs, or of PASA-comprising proteins, or both, are used in conjunction with the genetic code (Table 2) to design corresponding DNA sequences for the construction of synthetic oligonucleotides capable of specifically hybridizing

to genes encoding PASA-comprising proteins. Lathe, R., *J. Mol. Biol.* 183:1-12 (1985). The oligonucleotides are used as probes to detect DNA restriction fragments that encode PASA-comprising protein amino acid sequences; the restriction fragments are then cloned into appropriate vectors. Ruter, W.J., et al., U.S. Patent 4,440,859 (April 3, 1984). Alternatively, the oligonucleotides are used as primers in PCR reactions to amplify DNA sequences encoding PASA-comprising proteins. Mullis, K.B., et al., U.S. Patent 4,965,188 (October 23, 1990). In particular, inverse PCR is used to generate the maximum amount of PASA-comprising protein coding sequence information from the least amount of amino acid sequence information. Ochman, H., et al., in *PCR Technology: Principles and Applications for DNA Amplification*, Erlich, H.A., ed., Stockton Press, London, pages 105-111 (1989). The PCR products are then cloned into appropriate vectors and sequenced or sequenced directly. Gyllenstein, U., in *PCR Technology: Principles and Applications for DNA Amplification*, Erlich, H.A., ed., Stockton Press, London, pages 45-60 (1989). To produce PASA-comprising proteins, DNA sequences encoding the proteins are cloned into appropriate expression vectors which are then introduced into their cognate host cells, wherein the directed biosynthesis of PASA-comprising proteins occurs. Rutter, W.J., et al., U.S. Patent 4,440,859 (Apr. 3, 1984).

#### ***Example 8: Therapeutic Compositions***

The PASA-comprising proteins of the previous example, and the variable regions of the TCR subunits of the transgene of the invention, are used to generate several compositions useful in treating arthritic animals including humans.



### *Immunization with TCR Oligopeptides*

Although the mechanism remains unclear, immunization with synthetic oligopeptides corresponding to variable regions of TCR subunits has been demonstrated to prevent or reverse experimental allergic encephalomyelitis (EAE). EAE is induced by autoimmunization of animals with MBP and produces the clinical symptoms of MS, i.e., demyelination and paralysis. Howell, W.M., *et al.*, *Science* 246:668-670 (1989); Vanderbark *et al.*, *Nature* 341:541-544 (1989). A pilot trial of treatment of humans suffering from MS with TCR-derived oligopeptides is currently underway. Oksenberg, J.R., *et al.*, *J. Neurol. Sci.* 115(Suppl.):S29-S37 (1993).

Although the TCR variable region amino acid sequences of the invention are isolated from a murine hybridoma, the remarkable relatedness of TCRs involved in autoimmune diseases crosses species lines, suggesting that the molecular components of the immune system are sufficiently conserved so as to allow human therapeutic compositions to be derived from murine TCR molecules. For example, some MS patients have TCR populations that are over-represented by TCRs that recognize a fragment of myelin basic protein (MBP) bound to a part of the HLA receptor (the HLA-DR2 molecule). A sequence of three amino acids, apparently required to recognize the MBP:HLA-DR2 complex, was present in these TCRs. Oksenberg, J.R., *et al.*, *Nature* 362:68-70 (1993). The same fragment of MBP is recognized by a prevalent class of TCRs in an animal model of MS, EAE. The TCRs that are prevalent in EAE animals have the same sequence of three amino acids found in TCRs in MS humans. Gold, D.P., *et al.*, *J. Exp. Med.* 174:1467-1476 (1991); Hashim, G., *et al.*, *J. Immunol.* 146:515-520 (1991); Offner, H., *et al.*, *J. Immunol.* 146:4165-4172 (1991); Vainiene, M., *et al.*, *J. Neurosci. Res.* 31:413-420 (1992); Offner, H., *et al.*, *J. Immunol.* 148:1706-1711 (1992); Gold, D.P., *et al.*, *J. Immunol.* 148:1712-1717 (1992); Offner, H., *et al.*, *J. Immunol.* 151:506-517 (1993).

The amino acid sequences of the variable regions of the TCR subunits derived from R28 [SEQ ID Nos. 5 and 7] are used to design synthetic oligopeptides having therapeutic value in non-murine animals including humans. Oligopeptides comprising amino acid sequences derived from SEQ ID NOs. 5 and 7 are chemically synthesized according to standard methods (Stewart and Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockland, Illinois (1985)) and purified from reaction mixtures by reversed phase HPLC. The most effective anti-arthritic oligopeptides are identified by the assays described in Example 4.

#### *Oral Tolerance Therapy*

Although autoimmunization can result from direct presentation of a self-antigen to an animal, e.g., by subcutaneous injection, tolerance can be achieved by presenting antigens to an animal by oral ingestion. This process, called oral tolerance therapy, appears to activate T cells that secrete cytokines that counter some of the autoimmune response's effects. Strobel, S., *et al.*, *Immunology* 56:577-564 (1985); Mowat, A.M., *Immunology* 56:253-260 (1985); Mowat, A.M., *et al.*, *Adv. Exp. Med. Biol.* 216A:709-720 (1987); Lamont, A.G., *et al.*, *Immunology* 63:737-739 (1988); Mowat, A.M., *et al.*, *Immunology* 64:141-145 (1988). An antigen presented by oral ingestion that results in enhanced tolerance of the antigen is called a tolerogen. Thompson, H.S., *et al.*, *Clin. Exp. Immunol.* 72:20-25 (1988); Thompson, H.S.G., and Staines, N.A., *Clin. Exp. Immunol.* 64:581-586 (1985); Thompson, H.S., and Staines, N.A., *Immunol. Today* 11:396-399 (1991).

Oral tolerance therapy for autoimmune disease was first demonstrated by feeding myelin basic protein to EAE animals. Bitar, D.M., and Whitacre, C.C., *Cell Immunol.* 112:364-370 (1988); Fuller, K.A., *et al.*, *J. Neuroimmunol.* 28:15-26 (1990); Whitacre, C.C., *et al.*, *J. Immunol.* 147:2155-2163 (1991). Oral tolerance therapy has been demonstrated to be effective in suppressing arthritis in animal models, particularly collagen-

induced arthritis (CIA). Thomsson, H.S., and Staines, N.A., *Clin. Exp. Immunol.* 64:581-586 (1986); Nagler-Anderson, C., *et al.*, *Proc. Natl. Acad. Sci. (USA)* 83:7443-7446 (1986). Synthetic oligopeptides, derived from the amino acid sequence of type II collagen, are also effective as tolerogens. Myers, L.K., *et al.*, *J. Exp. Med.* 170:1999-2010 (1989). Synthetic polypeptidic tolerogens can be manipulated to further define the epitope(s) required for tolerogenicity. Meyers, L.K., *et al.*, *J. Immunol.* 149:1439-1443 (1992); Meyers, L.K., *et al.*, *J. Immunol.* 150:4652-4658 (1993); Myers, L.K., *et al.*, *J. Immunol.* 151:500-505 (1993).

The amino acid sequences of oligopeptides bound to APCs in the transgenic arthritic mice of the invention, or of PASA-comprising proteins, are used to design the sequences of synthetic oligopeptides for oral tolerance therapy. For human therapy, amino acid sequences from human PASA-comprising proteins are preferably used. Oligopeptides comprising amino acid sequences of choice are chemically synthesized according to standard methods (Stewart and Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockland, Illinois (1985)) and purified from reaction mixtures by reversed phase HPLC. The most effective anti-arthritic oligopeptides are identified by the assays described in Example 4, using oral ingestion as the means of administering the oligopeptides to the arthritic animals.

### ***MHC Antagonists***

In EAE, *in vivo* competition between the artificially-introduced "self" antigen and synthetic oligopeptides derived therefrom can be used to modulate the induction of autoimmune responses. These MHC antagonists are apparently bound by MHC molecules of APCs and presented to T cells but, because of chemical changes from the original antigen, are not sufficiently stimulatory to T cells so as to activate them. Zamvil, S.S., and Steinman, L., *Annu. Rev. Immunol.* 8:579-621 (1990); Steinman, L., *Adv. Immunol.* 49:357-379. The activation of T cells responsible for subsequent immunologic events

that culminate in arthritis is delayed or inhibited, with a parallel effect on the course of the disease.

5 The amino acid sequences of oligopeptides bound to APCs in the transgenic arthritic mice of the invention, or of PASA-comprising proteins, are used to design the sequences of synthetic oligopeptides that serve as MHC antagonists. For human therapy, amino acid sequences from human PASA-comprising proteins are preferably used. Oligopeptides comprising amino acid sequences of choice are chemically synthesized according to standard methods (Stewart and Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., 10 Rockland, Illinois (1985)) and purified from reaction mixtures by reversed phase HPLC. The most effective anti-arthritic oligopeptides are identified by the assays described in Example 4.

### *Utility of the Invention*

15 The embodiments of the invention described above can be used for such purposes as listed below, alone or in combination with each other or other complementary methods and/or compositions.

(1) Potential anti-arthritic therapeutic compositions are evaluated for their efficacy by determining the effect of the compositions on the temporal and/or histological progress of arthritis in transgenic arthritic mice. 20 Potential anti-arthritic therapeutic compositions that are evaluated include, but are not limited to, chemical compounds, tolerogens, anti-inflammatory agents, genetic therapy agents, and genetically engineered microorganisms or cells. See Example 4 for details.

(2) Potential arthritogenic (arthritis-inducing) compositions are 25 evaluated for their hazardousness by determining the effect of the compositions on the temporal and/or histological progress of arthritis in transgenic arthritic mice, or by determining their ability to enhance penetrance of the KRN transgenic allele in non-arthritic KRN transgenic mice. Potential arthritogenic compositions that are evaluated include, but are not limited to,

chemical compounds, tolerogens, anti-inflammatory agents, genetic therapy agents, and genetically engineered microorganisms or cells. See Example 5 for details.

5 (3) B or T cells from arthritic transgenic mice are isolated and fused to immortalizing cells to generate B cell hybridomas that produce monoclonal antibodies that recognize an endogenous polypeptide arthritogenic self antigen (PASA), or T cell hybridomas that are activated by presentation thereto of PASA-comprising proteins. The hybridomas monoclonal antibodies are used (a) to evaluate individual animals including humans for their risk of  
10 developing rheumatoid arthritis and/or (b) to isolate PASA-comprising proteins from animals including humans. See Example 6 for details.

(4) Antigen-presenting cells (APCs) from transgenic arthritic mice are prepared *en masse* and polypeptide antigens associated therewith are isolated. Because of the limited TCR repertoire in the transgenic arthritic  
15 mice, most T cells are directed to endogenous polypeptide arthritogenic self antigens. Monoclonal or monospecific antibodies that recognize the APC-associated antigens are prepared and used to isolate PASA-comprising proteins from animals including humans. Additionally or alternatively, the amino acid sequences of APC-associated peptides are determined and are used to design  
20 oligonucleotide probes and/or primers that are used to isolate genes encoding PASA-comprising proteins, which are used to produce PASA-comprising proteins via recombinant DNA technology. See Examples 6 and 7 for details.

(5) Compositions, particularly synthetic oligopeptides, useful in anti-arthritic therapy, including oral tolerance therapy, in animals including  
25 humans, are derivable from the amino acid sequences of (a) isolated PASA-comprising proteins of the invention or (b) the variable regions of the TCR subunits of the invention [SEQ ID NOs. 5 and 7]. See Example 8 for details.

## SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Institut National de la Sante et de la Recherche Medicale  
Centre National de la Recherche Scientifique  
Universite Louis Pasteur, Strasbourg 1  
E.R Squibb & Sons, Inc.

(ii) INVENTORS: Benoist, C.  
Mathis, D.  
Kouskoff, V.

(iii) TITLE OF INVENTION: Transgenic Arthritic Mice

(iv) NUMBER OF SEQUENCES: 9

(v) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Sterne, Kessler, Goldstein & Fox  
(B) STREET: 1100 New York Avenue, Suite 600  
(C) CITY: Washington  
(D) STATE: D.C.  
(E) COUNTRY: U.S.A.  
(F) ZIP: 20005

(vi) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vii) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: to be assigned  
(B) FILING DATE: 18-MAY-1995  
(C) CLASSIFICATION:

(viii) PRIORITY APPLICATION INFORMATION:

(A) APPLICATION NO.: 08/246,242  
(B) FILING DATE: 19-MAY-1994  
(C) CLASSIFICATION:

(ix) ATTORNEY/AGENT INFORMATION:

(A) NAME: Goldstein, Jorge A.  
(B) REGISTRATION NUMBER: 29,021  
(C) REFERENCE/DOCKET NUMBER: 1383.008PC00

(x) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (202) 371-2600  
(B) TELEFAX: (202) 371-2540

## (2) · INFORMATION FOR SEO ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Lys Pro Val Asn Thr Phe Val His Glu Ser Leu Ala Asp Val Gln Ala  
1 5 10 15  
Val Cys Ser Gln Lys  
20

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 721 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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ATATTTAATC TATGAAATTT GTCCTTGCTA TAACACAGAT ATCCTCTTCT TCCTCCAGGG      180
ATGAGAAAGA CCCACGGAGG TTCAGTGACC CAGAAACAAG GTCAAGTGAC CCTTTCAGAA      240
GATGACTTCC TATTTATAAA TTGCACTTAT TCTACCACAA CGTACCCAAC TCTTTTCTGG      300
TATGTCCAAT ATCCTGGACA AGGTCCACAG CTCCTTCTGA AAGTCACAAC TGCCAACAAC      360
AAGGGAATCA GCAGAGGCTT TGAAGCTACA TATGAGAAAAG GGACCACGTC CTTCCACTTA      420
CAGAAAGCCT CAGTGCAGGA GTCAGACTCA GCCGTGTACT TCTGTGCTCT GGCCCCTTCC      480
AATACCAACA AAGTCGTCTT TGGAACAGGG ACCAGATTAC AAGTATTACC AAGTAAGTTC      540
TGAGACAGTG AAGCAATTTG AAAAGGTGTT TTCTATAAAT TCCAGCTTTG GCTGGATGTC      600
CAGGCCGCCC TTCCTAGCAC TGCATTCATT CTGCAGCTAC CCTCCATGCC TCTAGCTGTT      660
GTGTCCATGG GAGAGCAGGG TGTCAATTC CAGGTTAACC TTCTGTAAAC GCGGCCGCTT      720
A

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## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 583 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CTCGAGCCAA AGTATGAACA AGTGGGTTTT CTGCTGGGTA ACCCTTTGTC TCCTTACTGT      60
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CTGGGTAGCA GTGCCAAACC CCTTCCAGAC TGATTCTTTT TCTTTTCCAG AGACCACACA      180
TGGTGATGGT GGCATCATT CTCAGACACC CAAATTCCTG ATTGGTCAGG AAGGGCAAAA      240
ACTGACCTTG AAATGTCAAC AGAATTTCAA TCATGATACA ATGTACTGGT ACCGACAGGA      300

```

TTCAGGGAAA GGATTGAGAC TGATCTACTA TTCAATAACT GAAAACGGCG ATCTATCTGA	360
AGGCTATGAT GCGTCTCGAG AGAAGAAGTC ATCTTTTTCT CTCACGTGTA CATCTGCCCA	420
GAAGAACGAG ATGGCCCTTT TTCTCTGTGC CAGCAGTATA TCCACAAACA ACCAGGCTCC	480
GCTTTTTTGA GAGGGGACTC GACTCTCTGT TCTAGGTAAA CTATGGGACC AACTGGTGG	540
GACCATTGTC CTTTGGACCT GGAGTGTCTC TGTAACCCCG CGG	583

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 403 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: unknown  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..403

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATG AAC ACT TCT CCA GTT TTA GTG ACT GCG ATG CTG CTG TTC ATG CTT	48
Met Asn Thr Ser Pro Val Leu Val Thr Ala Met Leu Leu Phe Met Leu	
1 5 10 15	
GGG ATG AGA AAG ACC CAC GGA GGT TCA GTG ACC CAG AAA CAA GGT CAA	96
Gly Met Arg Lys Thr His Gly Gly Ser Val Thr Gln Lys Gln Gly Gln	
20 25 30	
GTG ACC CTT TCA GAA GAT GAC TTC CTA TTT ATA AAT TGC ACT TAT TCT	144
Val Thr Leu Ser Glu Asp Asp Phe Leu Phe Ile Asn Cys Thr Tyr Ser	
35 40 45	
ACC ACA ACG TAC CCA ACT CTT TTC TGG TAT GTC CAA TAT CCT GGA CAA	192
Thr Thr Thr Tyr Pro Thr Leu Phe Trp Tyr Val Gln Tyr Pro Gly Gln	
50 55 60	
GGT CCA CAG CTC CTT CTG AAA GTC ACA ACT GCC AAC AAC AAG GGA ATC	240
Gly Pro Gln Leu Leu Leu Lys Val Thr Thr Ala Asn Asn Lys Gly Ile	
65 70 75 80	
AGC AGA GGC TTT GAA GCT ACA TAT GAG AAA GGG ACC ACG TCC TTC CAC	288
Ser Arg Gly Phe Glu Ala Thr Tyr Glu Lys Gly Thr Thr Ser Phe His	
85 90 95	
TTA CAG AAA GCC TCA GTG CAG GAG TCA GAC TCA GCC GTG TAC TTC TGT	336
Leu Gln Lys Ala Ser Val Gln Glu Ser Asp Ser Ala Val Tyr Phe Cys	
100 105 110	
GCT CTG GCC CCT TCC AAT ACC AAC AAA GTC GTC TTT GGA ACA GGG ACC	384
Ala Leu Ala Pro Ser Asn Thr Asn Lys Val Val Phe Gly Thr Gly Thr	
115 120 125	
AGA TTA CAA GTA TTA CCA A	403
Arg Leu Gln Val Leu Pro	
130	



## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Met Asn Thr Ser Pro Val Leu Val Thr Ala Met Leu Leu Phe Met Leu
 1           5           10           15
Gly Met Arg Lys Thr His Gly Gly Ser Val Thr Gln Lys Gln Gly Gln
          20           25           30
Val Thr Leu Ser Glu Asp Asp Phe Leu Phe Ile Asn Cys Thr Tyr Ser
          35           40           45
Thr Thr Thr Tyr Pro Thr Leu Phe Trp Tyr Val Gln Tyr Pro Gly Gln
          50           55           60
Gly Pro Gln Leu Leu Leu Lys Val Thr Thr Ala Asn Asn Lys Gly Ile
          65           70           75           80
Ser Arg Gly Phe Glu Ala Thr Tyr Glu Lys Gly Thr Thr Ser Phe His
          85           90           95
Leu Gln Lys Ala Ser Val Gln Glu Ser Asp Ser Ala Val Tyr Phe Cys
          100          105          110
Ala Leu Ala Pro Ser Asn Thr Asn Lys Val Val Phe Gly Thr Gly Thr
          115          120          125
Arg Leu Gln Val Leu Pro
          130

```

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 462 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: unknown  
 (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1..462  
 (D) OTHER INFORMATION: /label= peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

ATG AAC AAG TGG GTT TTC TGC TGG GTA ACC CTT TGT CTC CTT ACT GTA      48
Met Asn Lys Trp Val Phe Cys Trp Val Thr Leu Cys Leu Leu Thr Val
 1           5           10           15
GAG ACC ACA CAT GGT GAT GGT GGC ATC ATT ACT CAG ACA CCC AAA TTC      96
Glu Thr Thr His Gly Asp Gly Gly Ile Ile Thr Gln Thr Pro Lys Phe
          20           25           30

```

CTG ATT GGT CAG GAA GGG CAA AAA CTG ACC TTG AAA TGT CAA CAG AAT Leu Ile Gly Gln Glu Gly Gln Lys Leu Thr Leu Lys Cys Gln Gln Asn	144
35 40 45	
TTC AAT CAT GAT ACA ATG TAC TGG TAC CGA CAG GAT TCA GGG AAA GGA Phe Asn His Asp Thr Met Tyr Trp Tyr Arg Gln Asp Ser Gly Lys Gly	192
50 55 60	
TTG AGA CTG ATC TAC TAT TCA ATA ACT GAA AAC GGC GAT CTA TCT GAA Leu Arg Leu Ile Tyr Tyr Ser Ile Thr Glu Asn Gly Asp Leu Ser Glu	240
65 70 75 80	
GGC TAT GAT GCG TCT CGA GAG AAG AAG TCA TCT TTT TCT CTC ACT GTG Gly Tyr Asp Ala Ser Arg Glu Lys Lys Ser Ser Phe Ser Leu Thr Val	288
85 90 95	
ACA TCT GCC CAG AAG AAC GAG ATG GCC CTT TTT CTC TGT GCC AGC AGT Thr Ser Ala Gln Lys Asn Glu Met Ala Leu Phe Leu Cys Ala Ser Ser	336
100 105 110	
ATA TCC ACA AAC AAC CAG GCT CCG CTT TTT GGA GAG GGG ACT CGA CTC Ile Ser Thr Asn Asn Gln Ala Pro Leu Phe Gly Glu Gly Thr Arg Leu	384
115 120 125	
TCT GTT CTA GGT AAA CTA TGG GAC CAA ACT GGT GGG ACC ATT GTC CTT Ser Val Leu Gly Lys Leu Trp Asp Gln Thr Gly Gly Thr Ile Val Leu	432
130 135 140	
TGG ACC TGG AGT GTC TCT GTA ACC CCG CGG Trp Thr Trp Ser Val Ser Val Thr Pro Arg	462
145 150	

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Asn Lys Trp Val Phe Cys Trp Val Thr Leu Cys Leu Leu Thr Val	
1 5 10 15	
Glu Thr Thr His Gly Asp Gly Gly Ile Ile Thr Gln Thr Pro Lys Phe	
20 25 30	
Leu Ile Gly Gln Glu Gly Gln Lys Leu Thr Leu Lys Cys Gln Gln Asn	
35 40 45	
Phe Asn His Asp Thr Met Tyr Trp Tyr Arg Gln Asp Ser Gly Lys Gly	
50 55 60	
Leu Arg Leu Ile Tyr Tyr Ser Ile Thr Glu Asn Gly Asp Leu Ser Glu	
65 70 75 80	
Gly Tyr Asp Ala Ser Arg Glu Lys Lys Ser Ser Phe Ser Leu Thr Val	
85 90 95	
Thr Ser Ala Gln Lys Asn Glu Met Ala Leu Phe Leu Cys Ala Ser Ser	
100 105 110	

Ile Ser Thr Asn Asn Gln Ala Pro Leu Phe Gly Glu Gly Thr Arg Leu  
 115 120 125  
 Ser Val Leu Gly Lys Leu Trp Asp Gln Thr Gly Gly Thr Ile Val Leu  
 130 135 140  
 Trp Thr Trp Ser Val Ser Val Thr Pro Arg  
 145 150

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTTGTGGTA TTGAAGGGG CCAGAGCACA GAAGTACACG

40

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Pro Thr Gly Pro Leu Gly Pro Lys Gly Gln Thr Gly Glu Leu Gly Ile  
 1 5 10 15  
 Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys  
 20 25

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A. The indications made below relate to the microorganism referred to in the description</b> on page <u>15</u> , line <u>18</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b>	
Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES	
Address of depositary institution (including postal code and country) INSTITUT PASTEUR 28, Rue du Docteur Roux F-75724 PARIS CEDEX 15 FRANCE	
Date of deposit 18 May 1994 (18.05.94)	Accession Number 1-1413
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
E.coli/paKRN	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
(Blank space for designated states)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
(Blank space for separate furnishing of indications)	

<b>For receiving Office use only</b> <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer <u>Sigfried Hostad</u> Sigfried Hostad International Division RO/US (703) 305-3682	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>15</u> , line <u>28</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES</b>	
Address of depositary institution (including postal code and country) <b>INSTITUT PASTEUR 28, Rue du Docteur Roux F-75724 PARIS CEDEX 15 FRANCE</b>	
Date of deposit <b>18 May 1994 (18.05.94)</b>	Accession Number <b>1-1414</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>E.coli/pbKRN</b>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<div style="text-align: right; font-size: small;">For receiving Office use only</div> <div style="margin-top: 10px;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="margin-top: 20px;">Authorized officer  <b>Sigfried Hostad International Division RO/US (703) 305-3682</b></div>	<div style="text-align: right; font-size: small;">For International Bureau use only</div> <div style="margin-top: 10px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="margin-top: 20px;">Authorized officer</div>
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***What Is Claimed Is:***

1. A transgenic arthritic mouse having a phenotype of chronic inflammation and deformation of one or more synovial joints, said phenotype being conferred by transgenes contained in the somatic and germ cells of said mouse, wherein said transgenes direct the expression of functionally rearranged T cell receptor  $\alpha$  and  $\beta$  subunits.

2. A transgenic mouse having transgenes contained in the somatic and germ cells of said mouse, wherein said transgenes direct the expression of T cell receptor  $\alpha$  and  $\beta$  subunits that combine to form a T cell receptor that recognizes

(a) a polypeptide comprising the amino acid sequence

Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-  
Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1];

(b) a protein comprising one or more endogenous polypeptide arthritogenic self antigens; or

(c) both the polypeptide of (a) and the protein of (b).

3. The mouse of claim 2, wherein said transgenes comprise

(a) a first DNA sequence encoding a T cell receptor  $\alpha$  subunit comprising the variable region from the functionally rearranged TCR $\alpha$  gene of hybridoma R28;

5 (b) a second DNA sequence encoding a T cell receptor  $\beta$  subunit comprising the variable region from the functionally rearranged TCR $\beta$  gene of hybridoma R28; and

(c) a means of expressing said first DNA sequence and said second DNA sequence *in vivo*.

10 4. A mouse having a phenotype of chronic inflammation and deformation of one or more synovial joints, said mouse produced by introducing a viral vector comprising

(a) a first DNA sequence encoding a T cell receptor  $\alpha$  subunit comprising the variable region from the functionally rearranged TCR $\alpha$  gene of hybridoma R28;

15 (b) a second DNA sequence encoding a T cell receptor  $\beta$  subunit comprising the variable region from the TCR $\beta$  gene of hybridoma R28; and

20 (c) a means of expressing said first DNA sequence and said second DNA sequence *in vivo*.

5. The mouse of either of claims 3 or 4, wherein

(a) said first DNA sequence is selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, and equivalents thereof;

(b) said second DNA sequence is selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, and equivalents thereof;

(c) said means of expressing said first DNA sequence consists essentially of the TCR V $\alpha$  promoter region from the functionally rearranged TCR $\alpha$  gene of hybridoma R28, and said means of expressing said second DNA sequence consists essentially of the TCR V $\beta$  promoter region from the functionally rearranged TCR $\beta$  gene of hybridoma R28.

6. The mouse of either of claims 3 or 4, wherein said mouse further comprises a Major Histocompatibility Complex locus that encodes an MHC molecule that displays one or more endogenous polypeptide arthritogenic self antigens.

7. The mouse of claim 6, wherein said Major Histocompatibility Complex locus is the Major Histocompatibility Complex locus from the NOD strain of mice.



8. The mouse of claim 5, wherein said mouse further comprises an Major Histocompatibility Complex locus that encodes an MHC molecule that displays one or more endogenous polypeptide arthritogenic self antigens.

5 9. The mouse of claim 8, wherein said Major Histocompatibility Complex locus is the Major Histocompatibility Complex locus from the NOD strain of mice.

10. A murine protein sharing a least one epitope with a polypeptide comprising the amino acid sequence

10 Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-  
Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1],

wherein said protein comprises a polypeptide arthritogenic self antigen in a transgenic arthritic mouse.

11. A mammalian protein sharing a least one epitope with a polypeptide comprising the amino acid sequence

15 Lys-Pro-Val-Asn-Thr-Phe-Val-His-Glu-Ser-Leu-Ala-  
Asp-Val-Gln-Ala-Val-Cys-Ser-Gln-Lys [SEQ ID NO. 1],

wherein said protein comprises a polypeptide arthritogenic self antigen in the mammalian species from which it is isolated and further comprises a contiguous stretch of at least 15 amino acids which is at least 90% identical to an amino acid sequence derived from the protein of claim 10.

- 5                   12.     The protein of claim 11, wherein said mammalian species is *Homo sapiens*.
13.     An isolated DNA sequence encoding the protein of any one of claims 10-12.
- 10               14.     A mouse having a phenotype of chronic inflammation and deformation of one or more synovial joints, said mouse produced by immunization at an early age with a composition comprising one or more synthetic oligopeptides derived from the amino acid sequence of the protein of any one of claims 10-12.
- 15               15.     A mouse having a phenotype of chronic inflammation and deformation of one or more synovial joints, said mouse produced by transfer of lymphocytes from the mouse of any one of claims 1-9 or 14.
16.     A method of producing a protein, comprising the step of culturing a microorganism containing an expression vector comprising the

isolated DNA sequence of claim 13 and a means of expressing said DNA sequence in said microorganism.

5 17. A method of producing a protein, comprising the step of culturing a viral expression vector comprising the isolated DNA sequence of claim 13 and a means of expressing said DNA sequence in host cells in which said viral expression vector is capable of reproducing.

10 18. A hybridoma cell produced by the fusion of an immortalizing cell with an antibody-producing cell isolated from a transgenic arthritic mouse, wherein said antibody-producing cell and said hybridoma cell produce an antibody that recognizes the protein of any one of claims 10-12.

19. A monoclonal antibody produced by the hybridoma cell of claim 18.

20. A monospecific antibody that recognizes oligopeptide antigens presented by antigen presenting cells of a transgenic arthritic mouse.

15 21. A method of determining if a animal, including a human, possesses an enhanced risk of developing rheumatoid arthritis comprising the step of immunoassaying a biological fluid from said animal with the monoclonal antibody of claim 19 or the monospecific antibody of claim 20.

22. A method of determining if an individual animal, including a human, possesses an enhanced risk of developing rheumatoid arthritis comprising the step of testing isolated T cells from an individual animal for T cell reactivity to the protein of any one of claims 10-12.

5           23. An affinity column comprising the monoclonal antibody of claim 19 or the monospecific antibody of claim 20.

10           24. A method of producing the protein of any one of claims 10-12 comprising the step of passing a biological sample from a animal, including a human, through the affinity column of claim 23, wherein said protein is retained by said column.

          25. A protein produced by a method comprising the step of passing a biological sample from one or more animals, including humans, through the affinity column of claim 23, wherein said protein

15           (a) is retained by said column; and  
          (b) comprises a polypeptide arthritogenic self antigen in the animal species from which said protein is isolated.

          26. The protein of claim 25, wherein said animal species is *Homo sapiens*.

27. A method for determining the anti-arthritic potential of a composition comprising the steps of:

(a) administering a known dose of said composition to a first transgenic arthritic mouse before inflammation, irremediable joint destruction and limb deformation have occurred;

(b) detecting the onset of irremediable joint destruction and limb deformation in said first transgenic arthritic mouse; and

(c) comparing the time of onset of irremediable joint destruction and limb deformation in said first transgenic arthritic mouse to the time of onset of irremediable joint destruction and limb deformation in a second transgenic arthritic mouse which has not been exposed to said composition,

wherein a statistically significant delay in the time of onset of irremediable joint destruction and limb deformation in said first transgenic arthritic mouse relative to the time of onset of irremediable joint destruction and limb deformation in said second transgenic arthritic mouse indicates the anti-arthritic potential of said composition.

28. A method for determining the anti-arthritic potential of a composition comprising the steps of:

(a) administering a known dose of said composition to a first transgenic arthritic mouse before irremediable joint destruction and limb deformation has occurred;

(b) monitoring the extent of irremediable joint destruction and limb deformation in said first transgenic arthritic mouse; and

(c) comparing the extent of irremediable joint destruction and limb deformation in said first transgenic arthritic mouse to the extent of irremediable joint destruction and limb deformation in a second transgenic arthritic mouse which has not been exposed to said composition,

wherein a statistically significant decrease in the extent of irremediable joint destruction and limb deformation in said first transgenic arthritic mouse relative to the extent of irremediable joint destruction and limb deformation in said second transgenic arthritic mouse indicates the anti-arthritic potential of said composition.

29. A method for determining the arthritogenic potential of a composition comprising the steps of:

(a) administering a known dose of said composition to a first KRN transgenic mouse before irremediable joint destruction and limb deformation has occurred;

(b) detecting the onset of irremediable joint destruction and limb deformation in said first KRN transgenic mouse; and

(c) comparing the time of onset of irremediable joint destruction and limb deformation in said first KRN transgenic mouse to the time of onset of irremediable joint destruction and limb

deformation in a second KRN transgenic mouse which has not been exposed to said composition,

wherein a statistically significant delay in the time of onset of irremediable joint destruction and limb deformation in said second KRN transgenic mouse relative to the time of onset of irremediable joint destruction and limb deformation in said first KRN transgenic mouse indicates the arthrogenic potential of said composition.

30. A method for determining the arthritogenic potential of a composition comprising the steps of:

(a) administering a known dose of said composition to a first KRN transgenic mouse before irremediable joint destruction and limb deformation has occurred;

(b) monitoring the extent of irremediable joint destruction and limb deformation in said first KRN transgenic mouse; and

(c) comparing the extent of irremediable joint destruction and limb deformation in said first KRN transgenic mouse to the extent of irremediable joint destruction and limb deformation in a second KRN transgenic mouse which has not been exposed to said composition,

wherein a statistically significant increase in the extent of irremediable joint destruction and limb deformation in said first KRN transgenic mouse relative to the extent of irremediable joint destruction and limb deformation in said

second KRN transgenic mouse indicates the arthrogenic potential of said composition.

5           31.    The method of any one of claims 27-30, wherein said composition comprises a chemical compound administered by circulatory injection or oral ingestion.

10           32.    The method of any one of claims 27-30, wherein said composition comprises a polypeptide administered by circulatory injection of an isolated or recombinant bacterium or virus that is live or attenuated, wherein said polypeptide is present on the surface of said bacterium or virus prior to injection.

15           33.    The method of any one of claims 27-30, wherein said composition comprises a polypeptide administered by circulatory injection of an isolated or recombinant bacterium or virus capable of reproduction within a mouse, and said polypeptide is produced within a mouse by genetic expression of a DNA sequence encoding said polypeptide.

          34.    The method of any one of claims 27-30, wherein said composition is selected from the group consisting of a prospectively anti-inflammatory composition, an actively anti-inflammatory composition, and a



composition that has no effect on inflammation *per se* but limits the destruction of tissue resulting from inflammatory events.

5           35.    The method of claim 29 or 30, wherein said first KRN transgenic mouse is a KRN-transgenic NOD mouse and said second KRN transgenic mouse is a KRN-transgenic NOD mouse.

36.    A method of delaying the onset of severe symptoms associated with rheumatoid arthritis in an individual demonstrating early signs of rheumatoid arthritis, comprising the step of administering via oral ingestion a synthetic oligopeptide derived from the protein of any one of claims 10-12.

10           37.    An isolated DNA sequence encoding a T cell receptor  $\alpha$  subunit comprising a variable region comprising the amino acid sequence of SEQ ID NO. 5.

15           38.    An isolated DNA sequence encoding a T cell receptor  $\beta$  subunit comprising a variable region comprising the amino acid sequence of SEQ ID NO. 7.

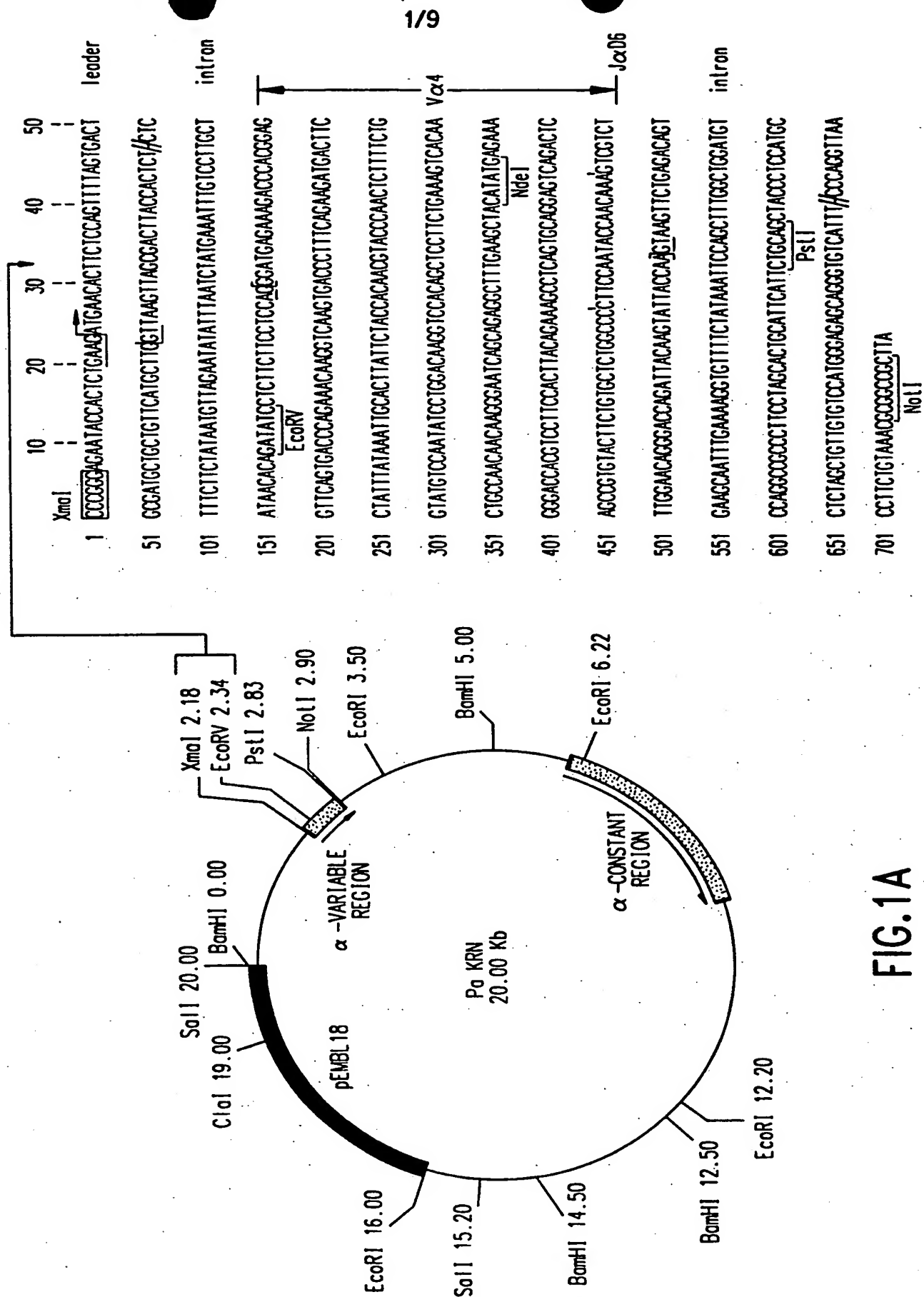
39.    A synthetic oligopeptide comprising at least 5 contiguous amino acids derived from the amino acid sequence of SEQ ID NO. 5.

40. A synthetic oligopeptide comprising at least 5 contiguous amino acids derived from the amino acid sequence of SEQ ID NO. 7.

41. An expression vector comprising the isolated DNA sequence of claim 13 and a means of expressing said DNA sequence *in vivo*.

5           42. An expression vector comprising the isolated DNA sequence of claim 37 or 38 and a means of expressing said DNA sequence *in vivo*.

10           43. The expression vector of claim 42, wherein said means of expressing said DNA sequence comprises the TCR V $\alpha$  promoter region from the functionally rearranged TCR $\alpha$  gene of hybridoma R28 or the V $\beta$  promoter region from the functionally rearranged TCR $\beta$  gene of hybridoma R28.



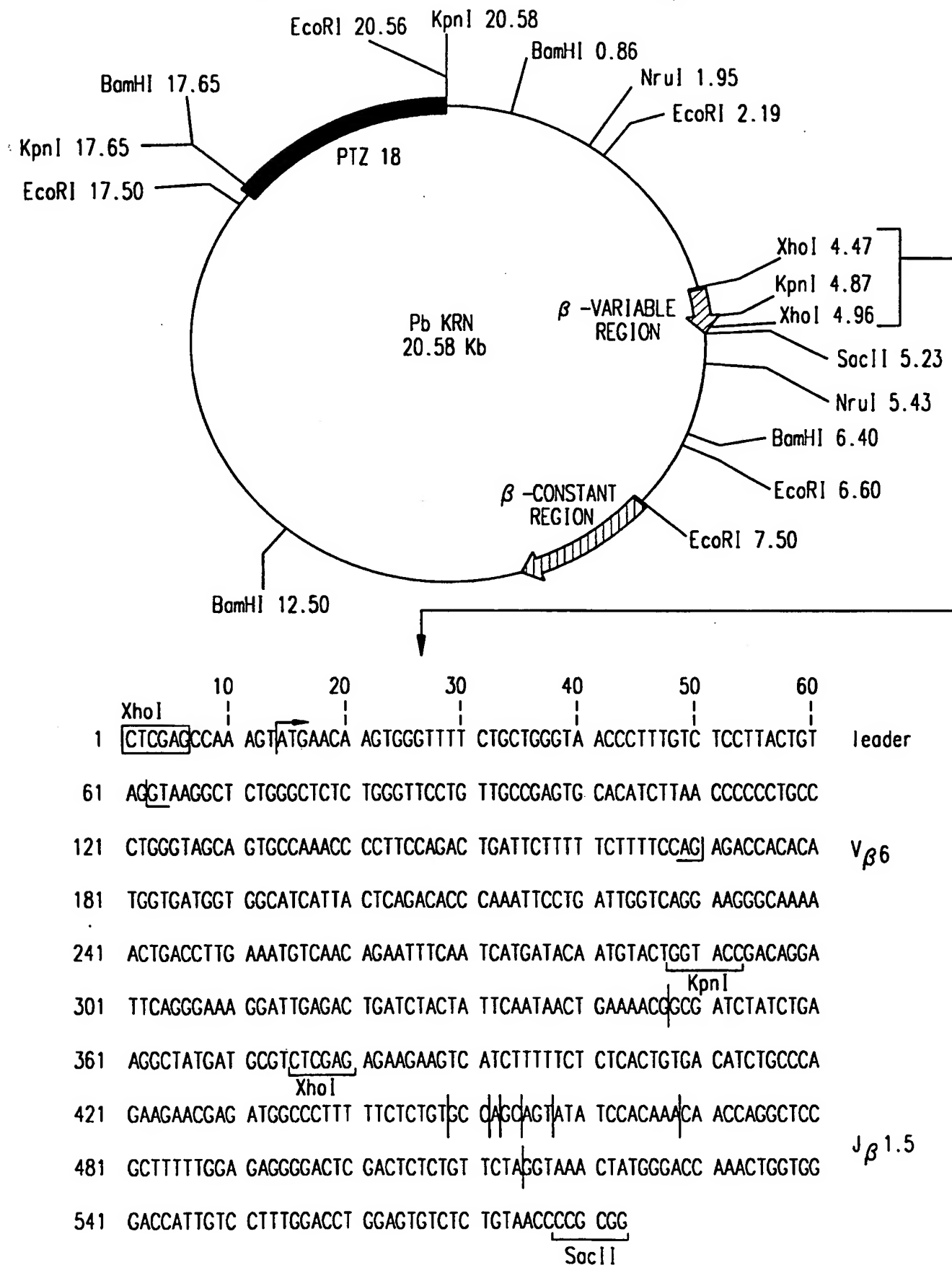


FIG.1B

SUBSTITUTE SHEET (RULE 26)

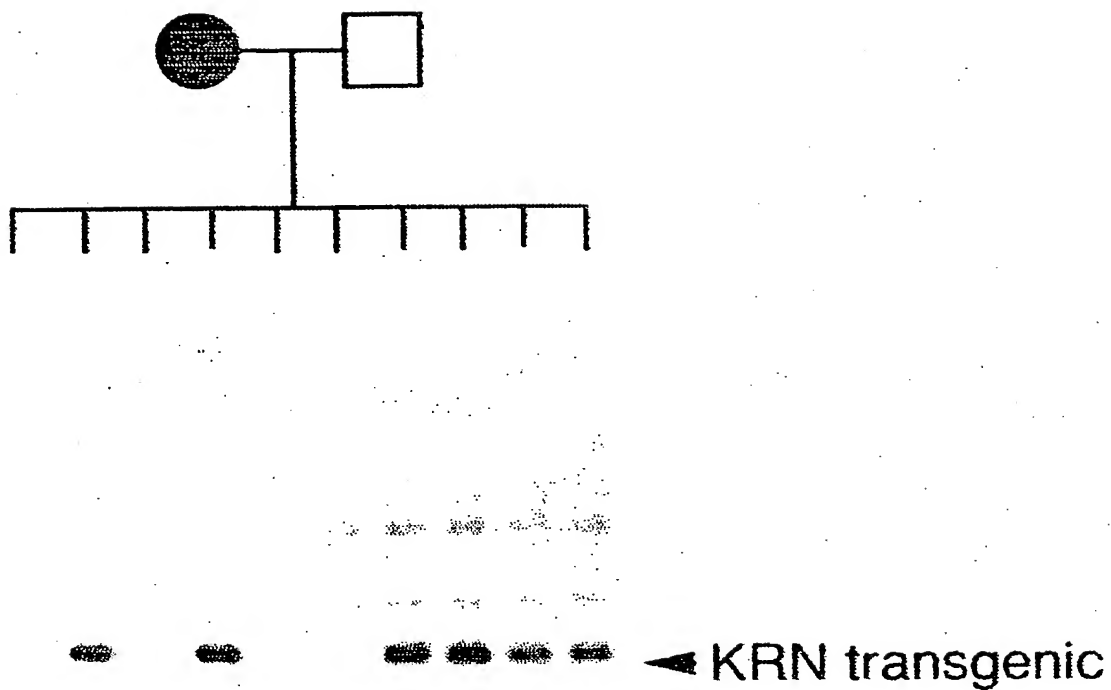


FIG.2

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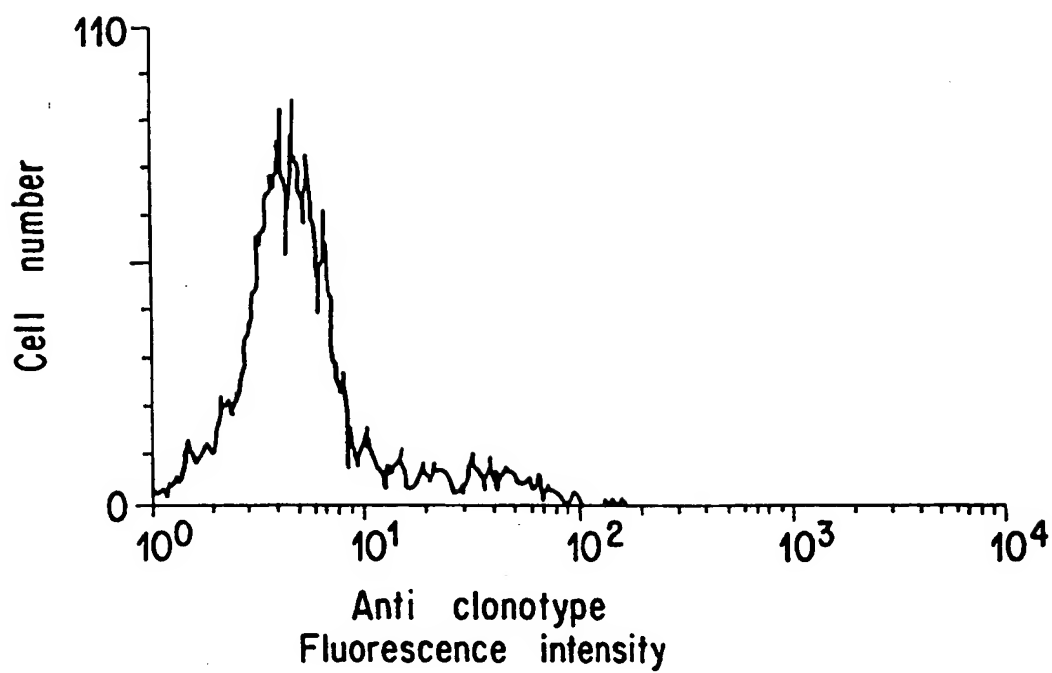


FIG. 3A

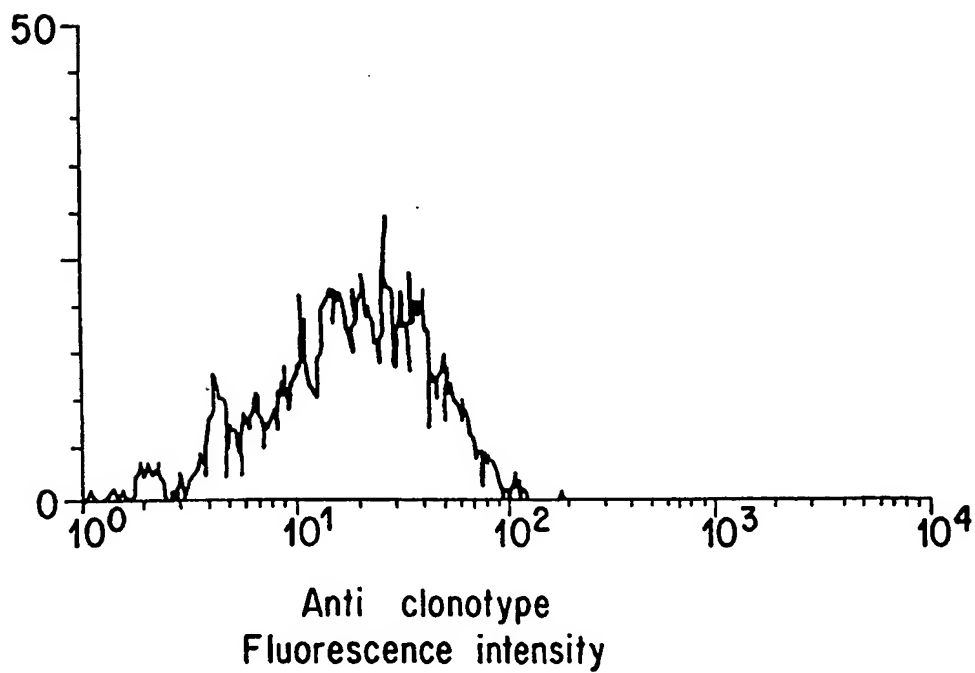


FIG. 3B

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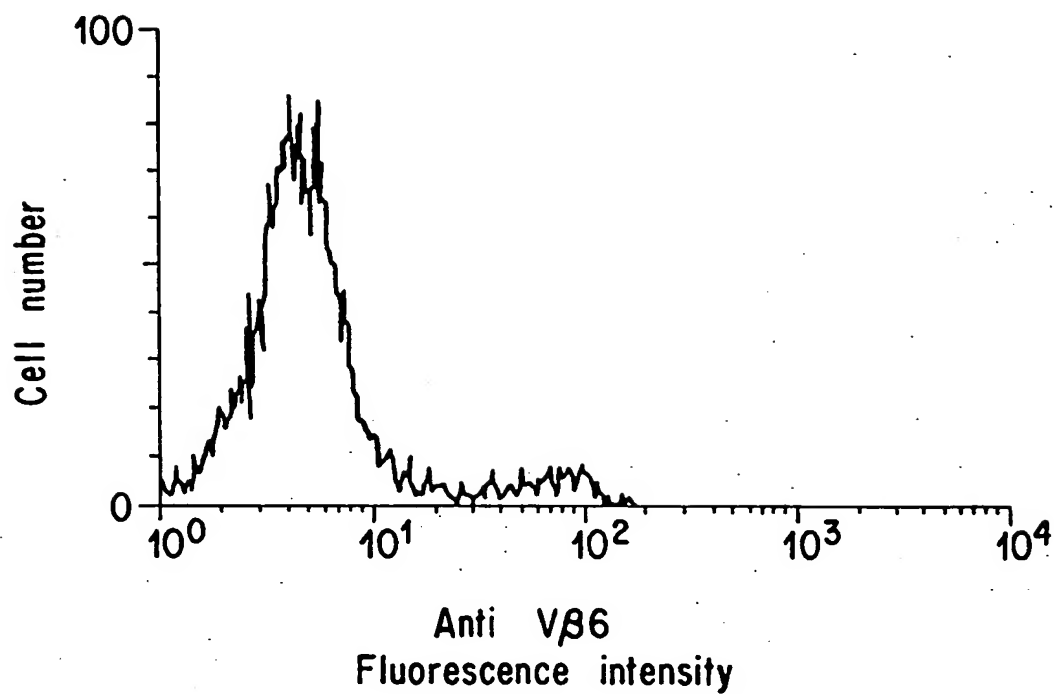


FIG. 3C

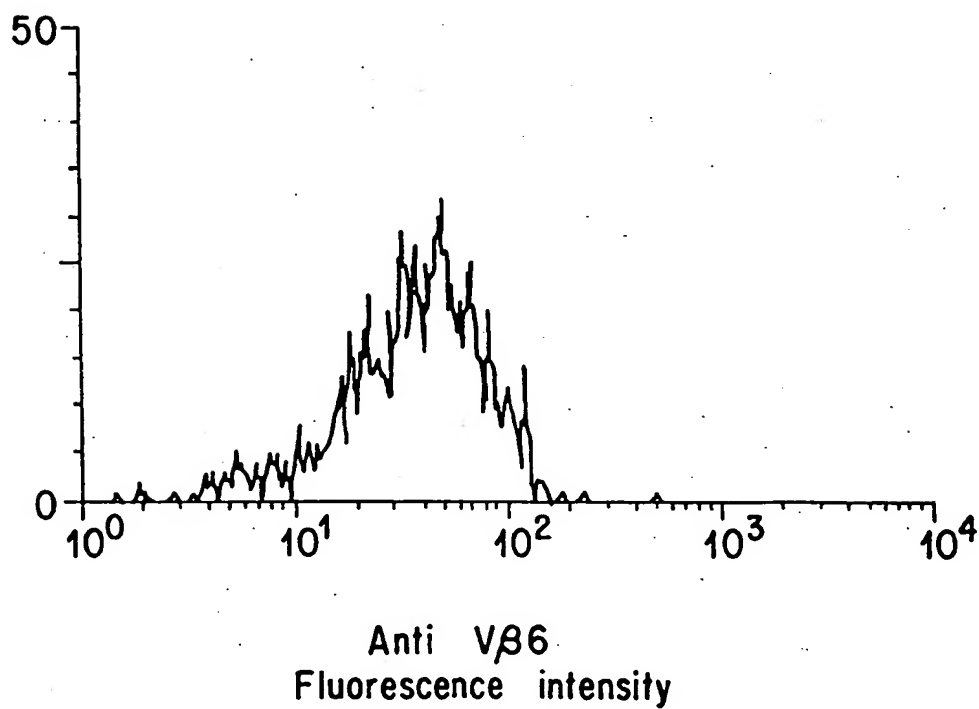


FIG. 3D

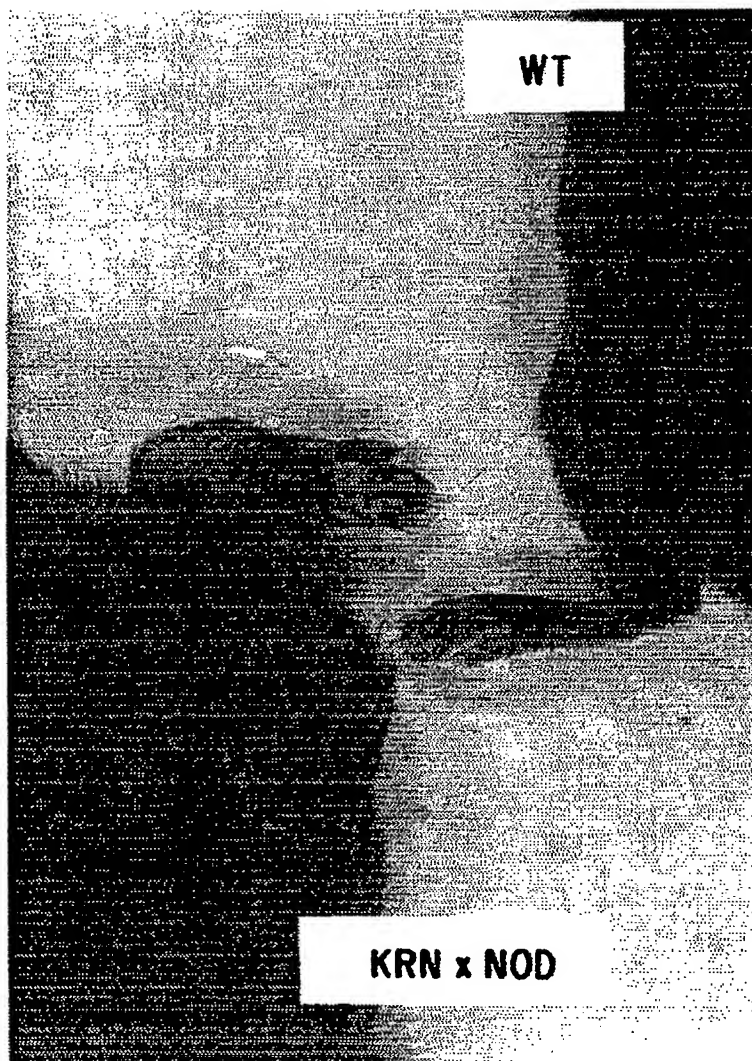


FIG.4A



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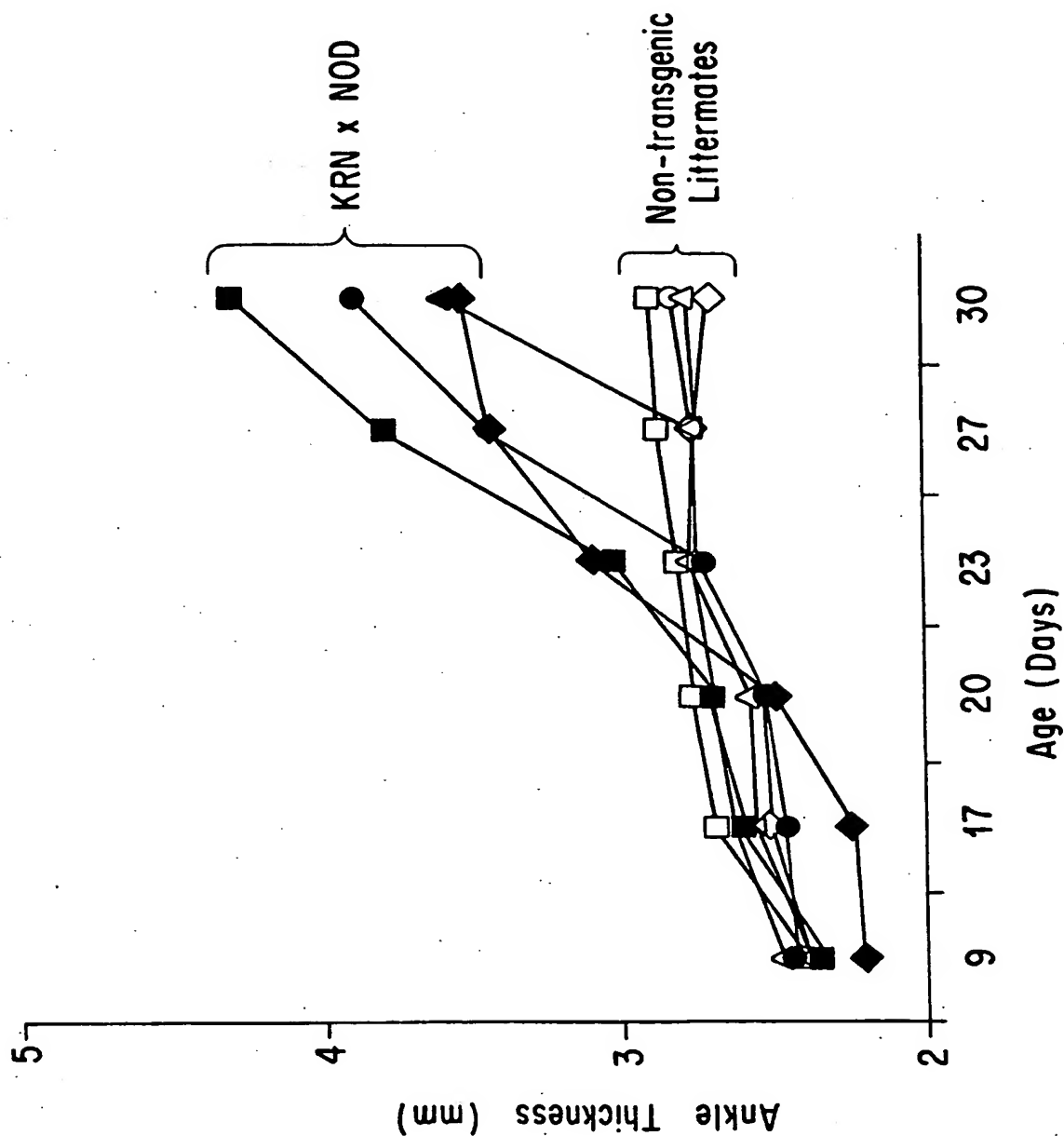


FIG. 4B

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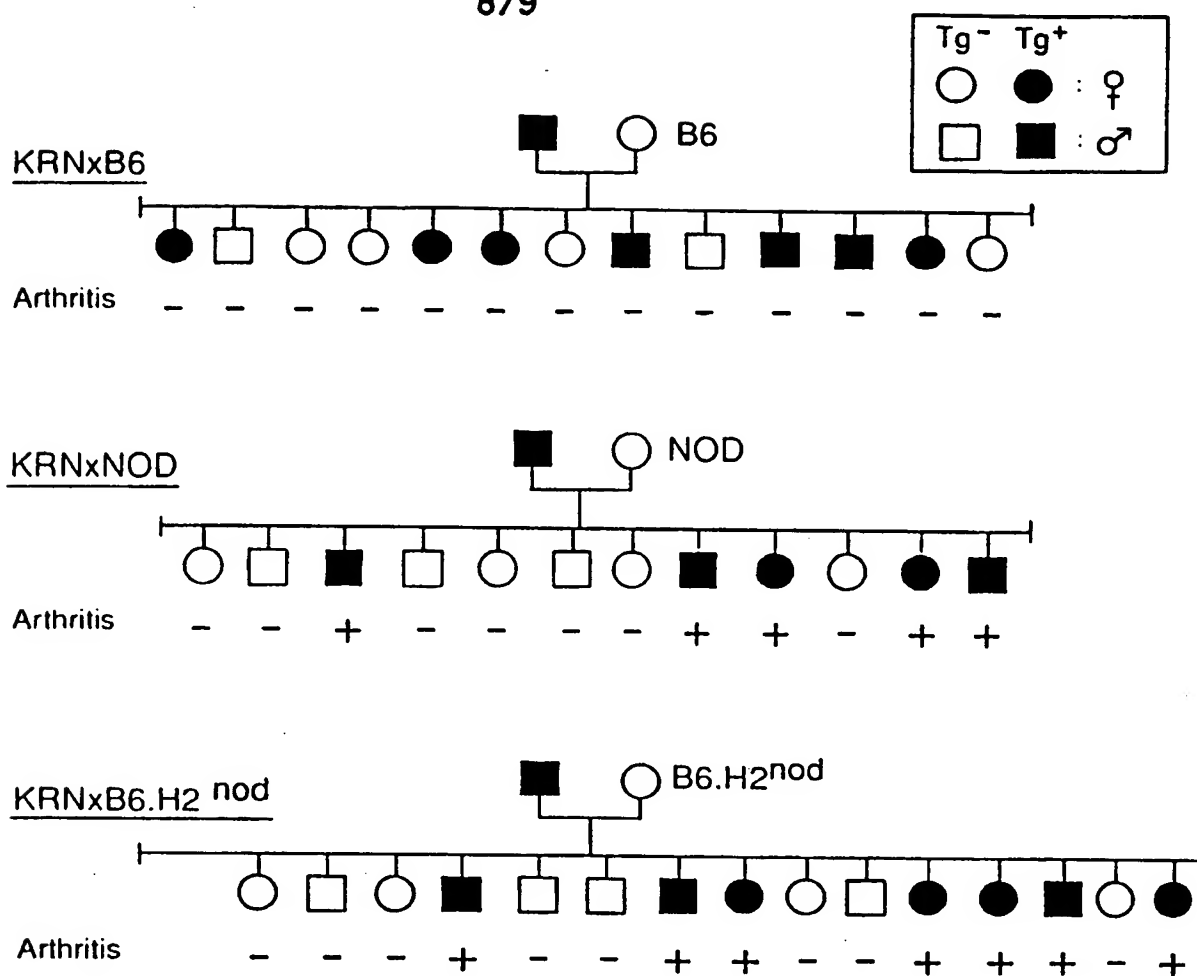


FIG. 5

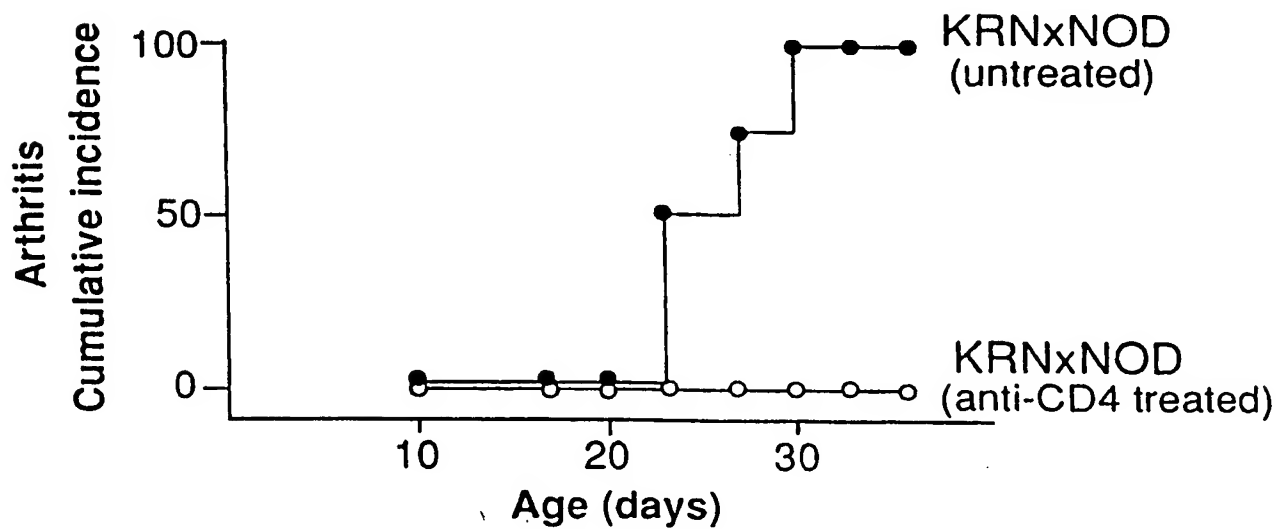


FIG. 7

SUBSTITUTE SHEET (RULE 26)

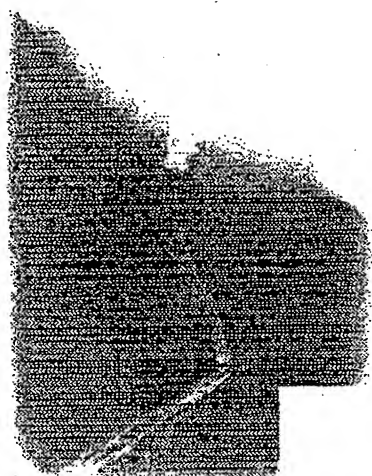


FIG. 6A

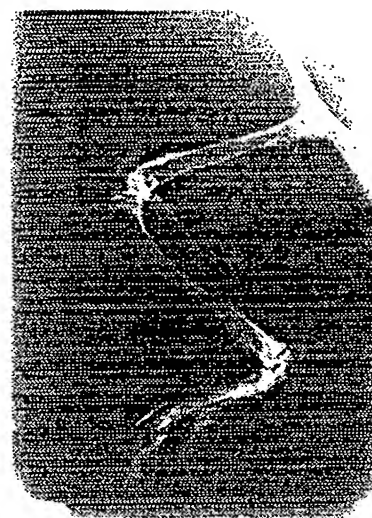


FIG. 6B

**(51) International Patent Classification <sup>6</sup> :**

**A3**

**(11) International Publication Number:**

**WO 95/32285**

**(43) International Publication Date:** 30 November 1995 (30.11.95)

**(21) International Application Number:** PCT/US95/05970

**(22) International Filing Date:** 18 May 1995 (18.05.95)

**(30) Priority Data:**

08/246.242

19 May 1994 (19.05.94)

US

**(71) Applicants:** INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM) [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cédex 13 (FR). CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS) [FR/FR]; 3, rue Michel-Ange, F-75794 Paris Cédex 16 (FR). UNIVERSITE LOUIS PASTEUR, STRASBOURG 1 [FR/FR]; 4, rue Blaise Pascal, Boîte postale 1032/F, F-67070 Strasbourg Cédex (FR). E.R. SQUIBB & SONS, INC. [US/US]; Lawrenceville-Princeton Road, Princeton, NJ 08543-4000 (US).

(72) **Inventors:** BENOIST, Christophe, O.; 2, rue des Hallebardes, F-67000 Strasbourg (FR). MATHIS, Diane, J.; 2, rue des Hallebardes, F-67000 Strasbourg (FR). KOUSKOFF, Valerie; National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206 (US).

(74) Agents: GOLDSTEIN, Jorge, A. et al.; Sterne, Kessler, Goldstein & Fox, Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).

(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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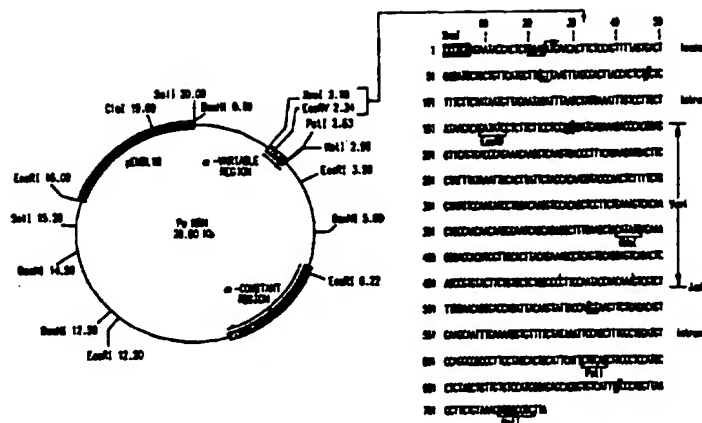
**With international search report.**

**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.**

**(88) Date of publication of the international search report:**

**15 February 1996 (15.02.96)**

**(54) Title: TRANSGENIC ARTHRITIC MICE**



**(57) Abstract**

A transgenic animal model for arthritis is disclosed. The arthritic condition results from genetic (or immunologic) manipulations that result in the T cell receptor (TCR) repertoire of the animal being substantially limited relative to the TCR repertoire of a wildtype control animal. The TCR repertoire of the arthritic animal, albeit limited, is functionally viable. In a preferred embodiment, the invention relates to transgenic arthritic mice wherein arthritis results from (1) a transgenic allele which encodes and expresses TCR  $\alpha$  and  $\beta$  subunits that combine in T lymphocytes of the transgenic animal to form a TCR that recognizes an antigen comprising one or more epitopes of an oligopeptide derived from amino acids 41-61 of bovine pancreatic ribonuclease and/or (2) polypeptide arthritogenic self antigens derived from endogenous proteins. The transgenic arthritic mice of the invention provides an animal model which faithfully mimics rheumatoid arthritis and by which human arthritogenic and therapeutic anti-arthritic compositions are evaluated. Also provided herein are therapeutic oligopeptides derived from the variable regions of the TCRs of the transgenes of the invention and/or from the amino acid sequence of proteins comprising endogenous polypeptide arthritogenic antigens.

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## INTERNATIONAL SEARCH REPORT

 Internal Application No  
 PCT/US 95/05970

 A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 6 C12N15/12 A01K1/027 C07K14/725 C12N9/22 C12N15/55  
 C12N5/18 C12P21/08 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A01K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF IMMUNOLOGICAL METHODS, vol.180, no.2, 27 March 1995, NEW YORK US pages 273 - 280 KOUSKOFF, V. ET AL. 'Cassette vectors directing expression of T cell receptor genes in transgenic mice' see the whole document ---	37, 38, 42, 43
A	CELL., vol.74, 24 September 1993, CAMBRIDGE, MA US pages 1089 - 1100 KATZ, J.D. ET AL. 'Following a diabetogenic T cell from genesis through pathogenesis' see the whole document --- -/--	1, 6, 7

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

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- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- '&' document member of the same patent family

Date of the actual completion of the international search

11 September 1995

Date of mailing of the international search report

27.12.95

Name and mailing address of the ISA

 European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+ 31-70) 340-3016

Authorized officer

CHAMBONNET, F

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NATURE., vol.334, 14 July 1988, LONDON GB pages 156 - 159 BLÜTHMANN, H. ET AL. 'T-cell-specific deletion of T-cell receptor transgenes allows functional rearrangement of endogenous alpha-and beta-genes' cited in the application see the whole document ---</p>	1
A	<p>CELL., vol.69, 1 May 1992, CAMBRIDGE, MA US pages 529 - 537 BORGULYA, P. ET AL. 'Exclusion and inclusion of alpha and beta T cell receptor alleles' cited in the application see the whole document ---</p>	1
A	<p>WO,A,90 06359 (GENPHARM INTERNATIONAL) 14 June 1990 see the whole document ---</p>	1
A	<p>WO,A,92 07070 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 30 April 1992 see claims; example 2 ---</p>	6
A,P	<p>JOURNAL OF IMMUNOLOGY., vol.153, no.6, 15 September 1994, BALTIMORE US pages 2758 - 2768 GRIFFITHS, M.M. ET AL. 'Collagen-induced arthritis and TCRs in SWR and B10.Q mice expressing an E1 alpha transgene' see the whole document ---</p>	1
A	<p>WO,A,89 04837 (CNRS INSTITUT PASTEUR DE LILLE, INSTITUT PASTEUR) 1 June 1989 see table III ---</p>	2
A	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol.89, 1992, WASHINGTON US pages 8874 - 8878 NATARAJAN, R. ET AL. 'Major histocompatibility complex determinants select T-cell receptor alpha chain variable region dominance in a peptide specific response' see the whole document ---</p>	37
	<p>---</p>	

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## INTERNATIONAL SEARCH REPORT

Internat 1 Application No

PCT/US 95/05970

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF IMMUNOLOGY., vol.138, no.11, 1 June 1987, BALTIMORE US pages 3991 - 3995 SPINELLA, D.J. ET AL. 'Receptor diversity of insulin-specific T cell lines from C57BL (H-2b) mice' see the whole document ---	38
A	EMBO JOURNAL., vol.9, 1990, EYNSHAM, OXFORD GB pages 4215 - 4223 PECCOUD, J. ET AL. 'Delineation of antigen contact residues on an MHC class II molecule' cited in the application see the whole document ---	1
A	JOURNAL OF MOLECULAR EVOLUTION, vol.37, no.1, July 1993 pages 29 - 35 BREUKELMAN, H.J. ET AL. 'Sequences related to the ox pancreatic ribonuclease coding region in the genomic DNA of mammalian species' see the whole document ---	2
A	FEBS LETTERS., vol.41, no.2, 1 May 1974, AMSTERDAM NL pages 227 - 232 GAASTRA, W. ET AL. 'The primary structure of giraffe pancreatic ribonuclease' see the whole document ---	2
A	BIOCHEMICA ET BIOPHYSICA ACTA, vol.621, 1980 pages 89 - 103 BEINTEMA, J. J. 'Primary structures of pancreatic ribonucleases from bovidae. Impala, Thomson's gazelle, nilgai and water buffalo' -----	2



## INTERNATIONAL SEARCH REPORT

Int'l application No.

PCT/US95/05970

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 36  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although this claim is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- claims 1-9, 14, 15, 18-40, 42, 43
- claims 10-13, 16, 17, 41

./...

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.☒ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

## 1. Claims: 1-9,14,15,18-40,42,43

A transgenic arthritic mouse having a phenotype of chronic inflammation and deformation of one or more synovial joints, said phenotype being conferred by transgenes contained in the somatic and germ cells of said mouse, wherein said transgenes direct the expression of functionally rearranged T cell receptor  $\alpha$  and  $\beta$  subunits;

A hybridoma cell produced by the fusion of an immortalizing cell with an antibody-producing cell isolated from a transgenic arthritic mouse, the monoclonal antibody produced by the hybridoma cell;  
An affinity column comprising the monoclonal antibody  
A method of producing the protein comprising the step of passing a biological sample from a animal, including human, through the affinity column.

A method of determining if a animal, including a human, possesses an enhanced risk of developing rheumatoid arthritis comprising the step of immunoassaying a biological fluid from said animal with the monoclonal antibody or the corresponding polypeptide;

A method of delaying the onset of severe symptoms associated with rheumatoid arthritis in an individual comprising the step of administering a synthetic oligopeptide derived from the protein, methods for determining the anti-arthritic potential of a composition: isolated DNA sequences encoding a T cell receptor  $\alpha$  or  $\beta$  subunit comprising a variable region comprising the amino acid sequence of SEQ ID 5 or 7, An expression vector comprising the isolated DNA sequence synthetic oligopeptides comprising at least 5 contiguous amino acids derived from the amino acid sequence

## 2. Claims: 10-13,16,17,41

A mammalian protein sharing at least one epitope with a polypeptide comprising the amino acid sequence SEQ ID no 1, DNA encoding it, expression vector comprising the isolated DNA sequence, method of producing the protein, by culture of host cells containing such a vector.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Internat J Application No

PCT/US 95/05970

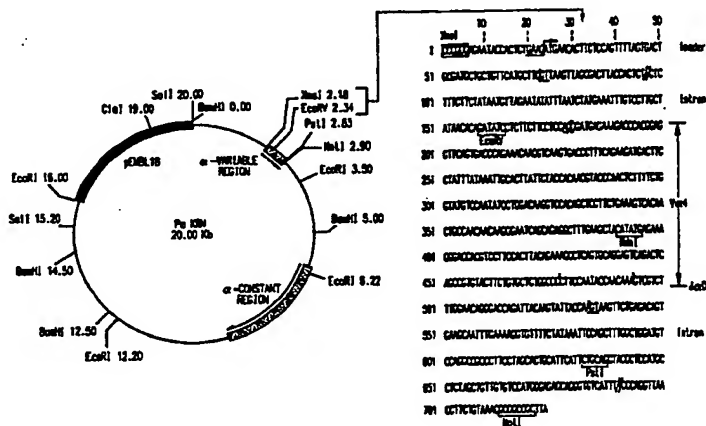
Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9006359	14-06-90	US-A- 5175384	29-12-92
		AU-B- 4755790	26-06-90
		CA-A- 2004477	05-06-90
		US-A- 5434340	18-07-95
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WO-A-9207070	30-04-92	AU-B- 8923191	20-05-92
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		AU-B- 3370189	01-11-90
		EP-A- 0342219	23-11-89
		US-A- 5171845	15-12-92
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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : C12N 15/12, A01K 67/027, C07K 14/725, C12N 9/22, 15/55, 5/18, 21/08, G01N 33/50		A3	(11) International Publication Number: <b>WO 95/32285</b>
(21) International Application Number: PCT/US95/05970		(43) International Publication Date: 30 November 1995 (30.11.95)	
(22) International Filing Date: 18 May 1995 (18.05.95)		(74) Agents: GOLDSTEIN, Jorge, A. et al.; Sterne, Kessler, Goldstein & Fox, Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).	
(30) Priority Data: 08/246,242 19 May 1994 (19.05.94) US		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(71) Applicants: INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cédex 13 (FR). CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE [FR/FR]. 3, rue Michel-Ange, F-75794 Paris Cédex 16 (FR). UNIVERSITE LOUIS PASTEUR, STRASBOURG 1 [FR/FR]; 4, rue Blaise Pascal, Boîte postale 1032/F, F-67070 Strasbourg Cédex (FR). E.R SQUIBB & SONS, INC. [US/US]; Lawrenceville-Princeton Road, Princeton, NJ 08543-4000 (US).		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(72) Inventors: BENOIST, Christophe, O.; 2, rue des Hallebardes, F-67000 Strasbourg (FR). MATHIS, Diane, J.; 2, rue des Hallebardes, F-67000 Strasbourg (FR). KOUSKOFF, Valerie; National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206 (US).		(88) Date of publication of the international search report: 15 February 1996 (15.02.96)	

(54) Title: TRANSGENIC ARTHRITIC MICE



## (57) Abstract

A transgenic animal model for arthritis is disclosed. The arthritic condition results from genetic (or immunologic) manipulations that result in the T cell receptor (TCR) repertoire of the animal being substantially limited relative to the TCR repertoire of a wildtype control animal. The TCR repertoire of the arthritic animal, albeit limited, is functionally viable. In a preferred embodiment, the invention relates to transgenic arthritic mice wherein arthritis results from (1) a transgenic allele which encodes and expresses TCR  $\alpha$  and  $\beta$  subunits that combine in T lymphocytes of the transgenic animal to form a TCR that recognizes an antigen comprising one or more epitopes of an oligopeptide derived from amino acids 41-61 of bovine pancreatic ribonuclease and/or (2) polypeptide arthritogenic self antigens derived from endogenous proteins. The transgenic arthritic mice of the invention provides an animal model which faithfully mimics rheumatoid arthritis and by which human arthritogenic and therapeutic anti-arthritic compositions are evaluated. Also provided herein are therapeutic oligopeptides derived from the variable regions of the TCRs of the transgenes of the invention and/or from the amino acid sequence of proteins comprising endogenous polypeptide arthritogenic antigens.

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